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BIOLOGICAL ACTIVITIES, PHENOLIC CONSTITUENTS AND OF VARIOUS EXTRACTS OF *CYCLAMEN COUM* TUBERS AND LEAVES FROM TURKEY

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The present study evaluated thephenolics composition and biological activities of various extracts of *Cyclamen coum.* The antioxidant properties of these plant wasdetermined using the scavengingmethods (DPPH andABTS), total antioxidant test systems (β-carotene/linoleic acid), reducing power (FRAP) assays.The antibacterial effect was examined with MIC method. The phenolics were identified using HPLC and the brine shrimp lethality test was used to screen for possible toxicity. Larvicidal activity against *Cx. pipienswas* assessed.The highest phenolic compound was foundcinnamic acid with 411.6 μg/g. The highest total phenolic and flavonoid were found in the ethanolic extracts with 8.99±3.07 mgGAE/mL and 54.66±3.02 mgQE/g, respectively. The tuber-methanol extract exhibited the highest antioxidant capacity (β-carotene/linoleic acid test, 76.43±3.02%). The radical scavinging activity of the extracts varied from 129.74±0.02 (tuber ethanol) to 336.3±0.02 (tuber acetone) IC_{50} μg/mL (DPPH assay) and from 118.04±0.07 (tuber ethanol) to 321.96±0.08 (leaves acetone) IC_{50} μg/mL (ABTS assay). Very strong reduction of Gr (+) *S. aureus* growth were observed during incubation in tubermethanolic extracts (MIC was 56±0.03μg/mL). The brine shrimp lethality assay of tubermethanol extract has showed good cytotoxic with LC_{50} of 3.016 μg/mL. The tuber extract of *C. Coum* showed highest larvicidal activity against *Cx. pipiens* with value LC_{50} (296.80 μg/mL). *C.coum* could be a source to derive the potential antioxidant and cytotoxic agents. However, further studies are needed to determine the mechanism involved in the process.

Keywords: Cyclamen coum, antioxidant, antibacterial, HPLC, phenolic.

Introduction

Antioxidants have been recognized as potential therapeutics for preventing different human diseases. Human beings have used plants as medicine for diverse health issues for thousands of years. Plants are widely used in traditional medicine of different countries and are a source of many potent and powerful drugs [1]. Since ancient time human beings depend on plants for meeting various daily needs such as food, medicine and for construction and other purposes. Plants are considered as an integral part of daily life. Plant based medicines are gaining much importance nowadays because of some drawbacks that are associated with the use of modern medicines [2]. Plants are known to produce a diverse range of bioactive molecules, making them a rich source of different types of medicines. Higher plants, as sources of bioactive compounds, have continued to play dominant role in the maintenance of human health since ancient times [3].

The genus *Cyclamen*, which possesses geofit plant species, belongs to the family of Primulaceae. The genus *Cyclamen* comprises about 21 species, which are predominately distributed in Southern Europe, Western Asia, Northern Africa and around the Mediterranean. In Turkey, this genus is represented with 12 taxa, 5 of which are endemic [4]. Cyclamen plants are used as medicinal plants and also used as ornamental plants. Pharmacological investigations on the extracts or isolated saponins of *Cyclamen* spp. tubers exhibited in vitro cytotoxic, antimicrobial, analgesic and anti-inflammatory activities. Also, analgesic, anti-inflammatory and antimicrobial activities of some *Cyclamen* species such as *C. repandum* and *C. mirabile* have been reported [5].

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Early investigations on the different *Cyclamen* species resulted in the isolation of triterpenoid saponins, a piperidine alkaloid and sterols [6]. It is generally believed that plants that have more saponin content show good cytotoxic activity, that is there is a direct correlation between total saponin content and cytotoxic activity. There are some studies about the chemical composition of several *Cyclamen* species which contain some triterpene saponins and glycosides. *Cyclamen* tubers have toxic saponins in abundant amounts. Even the tubers have posinious saponin, wild boars look for to collect and eat them without any post effect [7].

In a study by Arslan et al., [8] *C. alpinum* tuber extract might have a potential not only to inhibit and/or induce the metabolism of certain co-administered drugs but also to influence the development of toxicity and carcinogenesis owing to the induction of the cytochrome P450-dependent drug-metabolizing enzymes.

Oz et al., [9] investigated the larvicidal activity against *Culex pipiens* L. (Diptera: Culicidae) of the ethanolic extracts of the tuber parts of *C. alpinum* and *C. mirabile* Hildebr. species. They showed that *C. mirabile* (86.2 ppm) was more active than *C. alpinum* (161.3 ppm) according to LC_{50} values.

In Gundoğan's [10] study, the antimicrobial activity of the *C. mirabile* and *C. alpinum* species (bacteria: *B. subtilis* and *E. coli*, fungi: *C.albicans*), was not found antimicrobial activity against bactericides, but 12 mm inhibition zone of antifungal activity. In the same study, *C. mirabile* and *C. alpinum* species were found to be more effective in acetone extracts (IC_{50}: 48.33 ppm) in the DPPH assay with petroleum ether and acetone extracts.

In Turkish folkloric medicine the tubers of *C. coum* var. *coum* are used as an ovule in their natural form after removal of the outer surface, against infertility [11]. Pharmacological investigations on the extracts or isolated saponins of *Cyclamen* spp. tubers exhibited in vitro cytotoxic and anti-inflammatory activities [5].

C. coum is a non-pharmacopoeial plant, it is not used in official medicine. However, *C. coum* is used in traditional medicine for the treatment of hemorrhages, ecchymosis, inflammations, hemorrhoids, and cancer [12]. The tubers were used in Turkish folkloric medicine for infertility treatment in women. Recent studies have shown that *C.coum* can be used as a perspective medicinal plant in clinical practice [13].

In this study, the antioxidant, phenolic contents, total phenolic content and flavonoid content, antibacterial, larvacidal, cytotoxic activitesof leaves and tuber extracts of *C.coum* are examined employing various *in vitro* assay systems in order to understand the usefulness of this plant as well as in medicine and pharmacology. This is the first study on the antioxidant capacity and biological activities of the *C.coum*species.

Experimental

1. Plant materialand preparation of plant extract.

Cyclamen coum Boiss. species were collected in the spring 2015 from Kötekli locality, near Muğla province, in Turkey andidentified from the book of Flora of Turkey [14]. Each parts (tubers and leaves) were dried at the shadow, room temperature and low humidity.

Dried plant parts (tuber 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 55 °C for 6 h [13]. 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.

2. Evaluation of total antioxidant properties

2.1 β-Carotene/linoleic acid assay. The β-carotene/linoleic acid assay was carried out using the methodreported by Amin and Tan [15] with slight modifications. 0.2 mL 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. BHT (Butylated hydroxy toluen) was used as standards.

3. Investigation of radical scavenging activity

3.1 DPPH free radical scavenging assay. The DPPH method was conducted according to the procedure of Wu et al. [16] 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. The results were expressed as IC_{50} (the concentration required to inhibit 50% of the DPPH).

3.2 ABTS radical cation scavenging assay. Experiments were performed according to Re et al. [17] 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. The results were assessed as IC_{50} values (concentration in μg/mL thatcause 50% inhibition of the ABTS radicals).

4. Ferric-reducing antioxidant power (FRAP) assay

The reducing power of the extracts was determined according to the method described by Oyaizu [18]. Different concentrations of the sample were mixed with 0.2 M phosphate buffer (2.5 mL) and 1% potassium ferricyanide (2.5 mL). The mixture was kept at 50 °C for 20 min. Trichloroacetic acid (2.5 mL,10%) was added to reaction mixture. An aliquot of the upper layer (2.5 mL) was combined with 2.5 mL distilled water and 0.5 mL of a 0.1% ferric chloride. The absorbances were measured after 10 min, at 700 nm. The increased absorbance of the reaction mixture suggests a high reducing power. All the tests were carried out in triplicate. The results were represented as the equivalents of the trolox graph.

5. Determination of the total phenolic and flavonoid content

The total phenolic content of extracts were determined with Folin- Ciocalteau reagent, according to the method of Slinkard and Singleton [19]. 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 was determined using the Dowd method as adapted by Arvouet-Grand et al. [20]. 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).

6. Quantification of phenolic compounds 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-dihydroxybenzoic, 4-dihydroxy, chlorogenic acid, vanilic acid, caffeic acid, p-coumaric acid, ferulic acid and cinnamic acidwere 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 per gram of the extract.

7. Antibacterial activity

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. 5 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 *C.coum* methanolic 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 [21]. One hundred and eighty-five microliter (185µl) 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.

8. 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 [22]. 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}.

9. Larvicidal activity of extracts against the larvae of Culex pipiens L. (Diptera: Culicidae)

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 activity of the extracts against *Cx. Pipiens* was assessed by using the method described by Cetin and Yanikoglu [23]. 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-hr 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.

10. 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 *C.coum.* 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 softwareand 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 $p<0.05$.

Results and discussion

1. Total antioxidant activity. In this study, the β-Carotene/linoleic acid test system was used forevaluate the inhibit linoleic acid oxidation of the plant. The total antioxidantcapacity in ethanol, methanol and acetone extracts from *C. coum* ranged from 60.54 to76.43%. As Table 1 shows, total antioxidant activity was highest in the tuber methanol extract and to belowest in the tuber acetone extract. In the this assay, linoleic acid produces hydroperoxides during incubation at 50 °C. The presence of hydroperoxides causes rapid discoloration of β-carotene.

2. Radical scavenging activity. In DPPH assay, the hydrogen atoms or electron donating ability of the extracts investigated was determined from the bleaching of purple-colored methanol solution of DPPH and BHA and Trolox wereused as standards. The stable radical 2,2-Diphenyl-1-picrylhydrazyl (DPPH) was used as a reagent in this spectrophotometric method. Recently, food technologists and researchers have widely use the DPPH reactionas away of evaluating the free radical scavenging activity of plant extracts and food material [24]. As the Table 1 shows, the tuber methanol extract showed a slightly higher activity than the tuber acetone extract. It is reported that the decrease in the absorbance of the DPPH radical caused by phenolic compounds was due to the reaction between antioxidant molecules and radicals, resulting in the scavenging of the radical by hydrogen donation, which is visualized as a discoloration from purple to yellow.

The ABTS assay is used to assess the relative ability of an antioxidantto scavenge the ABTS generated in the aqueous phase, and compare it with the Trolox (water soluble vitamin E analogue) and BHA Standard. The ABTS is produced by reacting a potent oxidizing agent (e.g. potassiumpersulfate) with the ABTS salt. The reduction of the blue-green ABTS radical colored solution by hydrogen donating antioxidant is measured at 734 nm. According to the results, the tuber ethanol extracth as the strongest ABTS radical cation scavenging activity with IC_{50} 118.04±0.07µg/mL compared with the other extracts.

3. Ferric-reducing antioxidant power (FRAP) assay. The FRAP value reflected the reducing power of the extract. During the FRAP assay, the extract reduced $Fe^{3+}$to Fe^{2+}.Reducingabilities of plants serve as an important indicator of its antioxidant activity. All extracts exhibited reducing power antioxidant activity. The highest FRAP in the C.coum extracts were determined 7.189±0.08 (tuber-methanol) trolox equivalent (mg/g).

4. Total Phenol Content and Total Flavonoid Content. The content of the total phenolic and flavonoids in the ethanol, methanol and acetone extracts of *C.coum* presented in Table 2. The total phenol content in the extracts ranged from 1.56±2.02 to 8.99±3.07 mgGAE/mL (Table 2). The highest total phenolic was measured in the leaves-ethanol extract (8.99±3.07 mgGAE/mL). These results suggest that higher levels of antioxidant activity were due to the presence of phenolic components [25]. The concentration of flavonoids in the extracts of *C.coum* ranged from 17.71±1.03 to 54.66±3.02 mgQE/g (Table 2). The concentration of flavonoids in the tuber-ethanol extract was the highest (54.66±3.02 mgQE/g). The higher concentration of phenols and flavonoids in the methanol extract could be the reason for the observed high antioxidant property.

5. Phenolic composition. The analysis of phenolic compounds is very challenging due to the great variety and reactivity of these compounds. On the other hand, phenolics are suitable compounds for analysis using modern separation and detection methods, such as hyphenated techniques of HPLC with mass spectrometry, ultraviolet-visible light or nuclear magnetic resonance spectroscopy. The results for the phenolic compounds are shown in Table 3. Cinnamic acid was detected to be the major phenolic component in extract, contributing about 411.6 µg/g. Phenolic acids, such as caffeic and ferulic acids, were indicated as antibacterial constituents [26].

6. Antibacterial activity. Antibacterial activity of methanol extracts was tested against two different bacteria, including one gram-positive bacteria (*S.aureus* ATCC 25923) and one gram-negative bacteria (*E.coli* ATCC 25922) and the results are presented in Table 4. Methanol extracts of the tested *C.coum* was evaluated as natural antibacterial agents against certain bacteria known to cause infection in humans. The MIC was used as a parameter of the significant inhibitory effects induced by *C.coum*extracts in the growth of the tested microorganisms. All extracts evidenced antibacterial activity, and showed different selectivity and MIC for each microorganism. Bacteria were inhibited by tuber extracts, at very low concentrations, presenting an MIC of <100 (µg/mL) forbacteria. The presence of flavonoid and/or phenolic in the plant species extracts might play a role in the observed antibacterial activity.

Table 1. Antioxidant properties of *C. coum* extracts

Extracts	DPPH*	ABTS*	FRAP (Trolox equivalent)* (mg/g)	β-caroten/linoleic acid assay* (%)
	IC_{50}, µg/mL			
Tuber Ethanol	129.74±0.02	118.04±0.07	5.543±0.02	66.43±2.04
Leaves Ethanol	164.8±0.04	132.34±0.05	5.487±0.07	70.58±1.03
Tuber Methanol	129.68±0.03	122.09±0.02	7.189±0.08	76.43±3.02
Leaves Methanol	247.2±0.05	265.76±0.04	5.826±0.05	65.71±0.06
Tuber Acetone	336.3±0.02	298.56±0.09	2.926±0.04	60.54±0.03
Leaves Acetone	257.6±0.02	321.96±0.08	1.094±0.06	64.58±0.07
BHA	19.86±0.04	12.05±0.02	10.054±0.03	95.78±0.01

* Data were given as the mean of the threemeasurements (n=3)±standard error. The letters after themean values in each columnrefers to statistically different than the others ($p<0.05$).

Table 2. Total phenolic and total flavonoid contents of *C. coum* extracts

Plant extracts	Total phenolic content (mg GAE/mL extract) *	Total flavonoid content (mgQE/g) *
Tuber Ethanol	4.69±3.02	54.66±3.02
Leaves Ethanol	8.99±3.07	44.38±2.04
Tuber Methanol	3.57±4.06	54.53±1.02
Leaves Methanol	8.84±4.01	43.75±2.01
Tuber Acetone	1.90±3.03	26.97±1.01
Leaves Acetone	1.56±2.02	17.71±1.03

* Data were given as the mean of the threemeasurements (n=3)±standard error. The letters after themean values in each columnrefers to statistically different than the others ($p<0.05$).

Table 3. Phenolic components in the ethanolic extracts of *C. coum*

Phenolic standard compounds	Standard retention time RT (min)	*C. coum* (µg/g)*
Gallic acid	7.8±0.00	119.6
3,4-dihydroxy benzoic acid (protokatekuik asit)	12.2±0.00	39.6
4-dihydroxy benzoic acid	16.9±0.00	344.1
Chlorogenic acid	19.4±0.00	67.9
Vanillic acid	21.7±0.00	42.4
Caffeic acid (3,4-dihydroxycinnamic acid)	24±0.00	34.3
p-coumaric acid	29.3±0.00	6.6
Ferulic acid	34.7±0.00	3.2
Cinnamic acid	70.7±0.00	411.6

* Based on dry weights.

Table 4. Antibacterial activity of the *C. coum*methanol extracts

Microorganism	Extract	MIC (µg/mL)*
S.aureus ATCC 25923	Leaves	105±0.02
	Tuber	56±0.03
E.coli ATCC 25922	Leaves	105±0.06
	Tuber	75±0.04

* Data were given as the mean of the threemeasurements (n=3)±standard error. The letters after themean values in each columnrefers to statistically different than the others ($p<0.05$).

7. 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 tuber methanol extract (CTM) of *C.coum*has showed good toxic to brine shrimp nauplii, with LC_{50} of 76.125 µg/mL. In addition, the degree of lethality was found to be directly proportional to the concentration of the extract (Fig.).

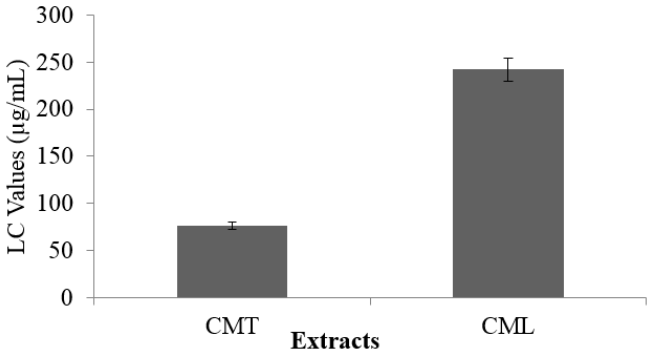
8. Larvicidal activity against Cx. Pipiens. Toxicities of methanol extracts from *C.coum* to young (second and third) instar *Cx. Pipiens* larvae were noted and the LC_{50} confidence limits for 24, 48 and 72 h were calculated (Table 5). All the extracts tested demonstrated significant larvicidal activity on *C. pipiens*, with LC_{50} values ranging from 1821.59 to 296.80 µg/mL. A concentration of 1000 µg/mL of the extract was found to be 80% larvicidal. The tuber extract of *C.coum* showed highest larvicidal activity against *Cx pipiens* with value LC_{50} (296.80 µg/mL) (Table 6).

Phenolic compounds could directly contribute to the antioxidant activity of the plants and thus the identification and measurement of phenolic compounds in plants is found out as one of the important tools in understanding the value of plants for human health.

Turan and Mammadov [27] have reported that the HPLC analyses reavealed that the phenolic contents of the ethanol extract contains predominatly *C. alpinum*ethanolic extract comprises phenolic compounds and 4-hydroxy benzoic acid was detected to be the major phenolic component in extract, contributing with 125.33 µg/g.

Dall'Acqua et al., [5] have reported that the phytochemical examination of the a new triterpene glycoside, repandoside was isolated together with six known saponins from the methanol extract of *C. repandum* tubers. The isolated saponins were characterized by high resolution mass spectrometry and both 1D and 2D NMR experiments.

The phenolics, such as flavonoids, phenolic acids and tannins are considered to be the major contributors to the antioxidant capacity [28]. The richness of the phenolic compounds in plants suggest their pharmacological properties; hence, analyses of the phenolic compounds in plants are crucial for understanding their medical value [29, 30].



Cytotoxics activity of methanol extracts by LC_{50} values. CMT: Methanol tuber extract of *C.coum* CML: Methanol leaves extract of *C.coum*

Table 5. Larvicidal activity the methanol extracts of *C. coum*against *Cx. pipiens* (% Mortality±SE)

Exposure times (h)		Test concentrations (ppm)*				
Extracts		0*	100	250	500	1000
Tuber	24	0±0.0	3.33±5.77	26.66±5.77	46.66±5.77	56.66±5.77
	48	1.3±1.3	26.66±5.77	36.66±5.77	56.66±5.77	60.0±10.0
	72	2.3±3.6	33.33±5.77	46.66±5.77	66.66±5.77	80.0±10.0
Leaves	24	1.3±1.3	0	3.33±5.77	23.33±3.33	30±0.004
	48	2.6±2.6	3.33±5.77	23.33±5.77	33.33±5.77	50±0.002
	72	3.6±3.6	13.33±5.77	30.0±0	43.33±5.77	76.66±5.77

*Control: Distilled water * Data were given as the mean of the three measurements (n=3)±standard error. The letters after the mean values in each column refers to statistically different than the others ($p<0.05$).

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

Extracts	Time (h)	LC_{50}(µg/mL)	LC_{90}(µg/mL)
Tuber	24	724.83	1595.10
	48	582.62	1797.57
	72	296.80	1250.09
Leaves	24	1821.59	2478.85
	48	1107.63	1967.36
	72	561.95	1411.91

Metin et al., [31] reported that total phenolic contents of *C. graecum* tubers and leaves. Results showed that the highest total phenolic contents was in the leaves-ethanol solution (33.73±0.69 µg of Pes) and the least was in the bulb-petroleum benzine solution (6.18±0.04 µg of Pes).

An-Najah National University (Nablus, Palestine) researchers evaluated the antibacterial and antioxidant activity of the methanol extract of the *C. coum* aerial parts due to the high content of flavonoids and phenols. Antioxidant activity (AO) was determined by spectrophotometry at a wavelength of 517 nm (AO=31 µg/mL). The MIC was determined by the method of serial dilution and its value was 6.25–12.5 mg/mL against various bacterial strains, including *S. aureus*, *E. coli*, *P. Aeruginosa* and methicillin-resistant *S. aureus* (MRSA). Higher activity was noted against *P. aeruginosa* (MIC=6.25 mg/ml), that affect the respiratory system, bones, joints, urinary tract, cause gastrointestinal infections, dermatitis, various systemic infections, especially in patients with severe burns [12].

In Gundoğan's [10] study, the antimicrobial activity of the *C. mirabile* and *C. alpinum* species (bacteria: *Bacillus subtilis* and *Escherichia coli*, fungi: *Candida albicans*), was not found antimicrobial activity against bactericides, but 12 mm inhibition zone of antifungal activity.

Okmen et al., [31] investigated theantibacterial effects of *C. mirabile* extracts against mastitis pathogens. The both of extract showed maximum inhibition zone against Coagulase-negative staphylococci-36 (CNS-36) and the zone was 12 mm. CNS-36 and CNS-37 showed the lowest sensitivity to *C. mirabile* ethanol extract (1625 µg/mL).

The brine shrimp cytotoxic bioassay is considered to be useful tool for the preliminary assessment of general toxicity and for estimating the medium lethality concentration (LC_{50}). In the toxicity evaluation of plant extracts using the brine shrimp lethality test, LC_{50} values lower than 1000 μg/mL are considered bioactive. Dusen et al., [32] investigated the cytotoxic activity of ethanolic extracts of eight *Cyclamen* taxa. Some of the extracts (LC_{50}<1000) were found to be active in the brine shrimp lethality bioassay. They showed that, all of the ethanolic extracts of the

tubers showed significant activity. Literature reports show that saponins, have been isolated from *Cyclamen* taxa extracts and found to be potentially toxic [32].

Important plant species used for different purposes in different parts of the world are spreading [33, 34]. The insecticidal activity of plant basedproducts (extracts) against different mosquito species has been evaluated by many authors. This study is the first to report on the larvicidal activity of the extracts of *C.coum* species against *Cx. pipiens*. Oz et al. [9] investigated the larvicidal activity against *Culex pipiens* L. (Diptera: Culicidae) of the ethanolic extracts of the tuber parts of *C. alpinum* and *C. mirabile* species. They showed that *C. mirabile* (86.2 ppm) was more active than *C. alpinum* (161.3 ppm) according to LC_{50} values.

Conclusions

In the present study, analysis of the phenolic component, antioxidant activity, cytotoxic activity, antibacterial activity and content of phenol, flavonoid revealed that the ethanol and methanol leaves, tuber extract of *C.coum* can be a potent source of natural antioxidants. The results of the present study demonstrated that the of *C.coum* constitute a rich source of antioxidants. The bioactive phytochemical compounds in *C.coum* were found to exhibit potential antioxidant activity through *in vitro* model. The high phenolic and flavonoid content and their significant linear correlation with antioxidant activity indicated that these compounds may be responsible for its antioxidant properties. The methanol extract also possessed significant amount of phenolic and flavonoids. The strong antioxidant and antibacterial potential of *C.coum* may be due to the presence of diverse phytochemical compounds, mainly flavonoids and phenolic acids. Further research is required to determine the potential health benefits of these bioactive components *in vivo* and elucidate their mechanism of action.

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