ANTIOXIDANT BIOCHEMICAL AND LARVICIDAL ACTIVITY OF *Cyclamen hederifolium* EXTRACTS

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ABSTRACT

This study aimed to determine the total phenolic and flavonoid contents of *Cyclamen hederifolium* ethanolic, methanolic and acetone extracts, and antioxidant activities of them, to investigate the potential safety/toxicity risks of the different parts and doses of the *C. hederifolium* ethanolic extracts using enzymes (ALT, ALP, and CRE), and the larvicidal effect of water extract against houseflies (*Musca domestica*) and mosquitoes (*Culex pipiens*). *In vitro* antioxidant activity of the extracts determined using DPPH and ABTS assays. Total phenolic and flavonoid contents of the extract represented as mg GAE/g-extract and mg QE/g-extract, respectively. For enzyme assay, the blood of five different groups of rats were analysed on days 0, 15, and 30. The highest value with the lowest IC_{50} value in the DPPH test was obtained from the ethanolic extract of the above ground (AG) (0.83 ± 0.00 µg/mL. The highest value with the lowest IC_{50} value in the ABTS test was obtained from the methanolic extract of the underground (UG) (0.16 ± 0.00 µg/mL). Statistically significant increases in AG 1% group and significant decreases in UG 1% group were observed in ALT and ALP values ($p \leq 0.05$). The AG extract was found to be more toxic against *M. domestica* (30.45 ± 2.78%) and *Cx. pipiens* (83.33 ± 0.00%). Considering the ALT and ALP measurements, the increase in the values in AG 1% group over time indicates liver damage. It seems that UG 1% extract reduces liver enzyme levels, and it has increased larvicidal effect over time.

KEYWORDS:
Antioxidant, *Cyclamen hederifolium*, Larvicidal Activity, Liver, Kidney

INTRODUCTION

Plants are inestimable value for many fields ranging from medicine, food, agriculture, and even the automotive industry, and are sources of chemical compounds containing a wide range of chemicals [1]. Secondary metabolites are complex chemicals in plants that have essential functions that occur during the synthesis of primary metabolites. Phytochemicals that are essential for plant life are synthesized with any stress factor [2]. These chemicals within the plant are affected by many factors and the growing seasons of plants, environmental conditions, and even among different species in the same genus may differ in the substances contained in it. Secondary metabolites are very little in the plant, as they are synthesized in the plant at certain cell types and at different growth times [3, 4]. Phytochemicals have a significant role in preventing diseases and improving health quality. Studies investigating many effects such as antioxidants, antimicrobials, larvicidal, pesticides, and herbicides that these materials have, are increasing day by day [5].

Synthetic insecticides/pesticides are accumulated in food, water, etc., and caused detrimental health effects on humans such as excessive tissue damage in liver and kidney tissues [6-8]. Enzyme levels of the serum are used as biochemical tools for detection of the liver and kidney injuries. While the elevation of the alanine aminotransferase (ALT) serve as a biomarker of the hepatocyte damage, increased level of the alkaline phosphatase (ALP) mostly related to the cholestasis. Also, creatinine (CRE) levels of in the serum used to diagnose kidney disease. Natural insecticides, such as plant secondary metabolites can be used to protect humans health and environment [9]. Nevertheless, these natural compounds can be toxic and they may have adverse effects on experimental animals [10].

Houseflies and mosquitoes are vectors that carry the mechanical carriers of many diseases that pose a severe threat to human and animal health [11]. Houseflies carry diseases such as typhoid fever, cholera, plague, leprosy, anthrax, and trachoma. The characteristic feature of 11 people who died in the deadly *Escherichia coli* bacteria strains in Japan in 1996 is that they are found in environments with a high density of houseflies [12]. The housefly reduction is tried to be done especially with synthetic pesticides such as diazinon, fenitrothion and pyrethrin

trough the continuous applications [13]. Among the mosquitoes, *Aedes*, *Culex* and *Anopheles* genera are the most important vectors that carry diseases such as malaria, filariasis, dengue, and yellow fever. It is estimated that 300-500 million people in the world are affected by the malaria disease that mosquitoes carry mechanically. Much stagnant water such as ornamental ponds in the cities and puddles in the excavations are suitable for the living of mosquito larvae, and the chemical medication of these environments is tried to be kept in balance with the mosquito population [14]. These synthetic chemicals cause resistance as a result of their regular use and should be used more against each resistance [13]. After each use, there are consequences such as leaving drug residues on ecological balance, a proliferation of unwanted species and attacking non-target organisms due to the reduction of fly predators. In this case, many harmful bacteria and viruses have the potential to enter human and animal life and cause significant dangers. It has the potential to enter human and animal life and cause very vital dangers. In recent years, research has shifted to secondary metabolites to the investigation of larvicidal, insecticidal, and herbicide properties due to such damages of synthetic drugs [15]. This study aimed to determine the total phenolic and flavonoid contents of *Cyclamen hederifolium* Aiton ethanolic, methanolic and acetone extracts, and the antioxidant activities of the extracts using DPPH and ABTS scavenging activity assays. This study was also aimed to investigate the potential safety/toxicity risks of the different parts and doses of the *C. hederifolium* ethanolic extracts using enzymes (ALT, ALP, and CRE), and the larvicidal effect of water extracts against houseflies (*Musca domestica*) and mosquitoes (*Culex pipiens*), and intended to provide some contributions to the industrial sectors such as pharmacy and agriculture.

MATERIALS AND METHODS

Chemicals, Plant Materials and Preparation of Extracts. Ethanol, methanol, acetone, DPPH, ABTS, trolox, sulfuric acid, Folin-Ciocalteu reagent, and butylated hydroxyanisole (BHA), was purchased from Sigma-Aldrich. Other chemicals and solvents were analytical grades. *Cyclamen* genus is included in the Primulaceae family. *C. hederifolium* was collected from Kuşadası, Aydın province, Turkey in February 2018 during the flowering period and identified according to Davis [16] and Güner *et al* [17]. Extracts were prepared according to the Yılmaz *et al.* [18] method, and were kept at -20 ^{o}C.

In vitro Antioxidant Activity and Determination of Contents. DPPH Free Radical Scavenging Activity. DPPH (2,2-Diphenyl-2-picrylhydrazyl radical) free radical scavenging activity was determined using Wu et al. method [19].

ABTS Radical Cation Scavenging Activity. ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)) radical cation scavenging activity was determined using Re *et al.* method [20]. According to this method, 7mM ABTS and 2.45 mM potassium persulfate were mixed and kept in the dark, at room temperature 12 hours before the assay. Then, it was diluted with ethanol until the absorbance was 0.700 at 734 nm in a spectrophotometer. 0.5 mg/mL extracts were mixed with 4.5 mL ABTS solution and incubated at room temperature for 30 minutes. Then measured at 734 nm. Ethanol was used as a blank solution. Results were calculated as the half-maximal inhibitory concentration (IC_{50}) values.

Determination of Total Phenolic Contents. The method which is developed by Singleton and Rossi [21] was used in order to determine the total phenolic contents. For this purpose, 1mg/mL extract, 1 mL Folin-Ciocalteu reagent and 46 mL distilled water mixed and sodium carbonate solution was added after 3 minutes, incubated in a dark place for 2 hours, and then the absorbance was measured at 760 nm. The total phenolic contents were represented as milligrams of gallic acid equivalents per gram of extract (mg GAE/g-extract), and determined by using the formula (1).

$$y = 0.0156x-0.0387 \quad (R^2=0.9861) \qquad (1)$$

Determination of Total Flavonoid Contents. The total flavonoid contents in *C. hederifolium* extract was determined as described by Arvouet-Grand *et al.* [22] and expressed in quercetin equivalents (mg QEs/g extract). $AlCl_3$ solution prepared in 1.0 mL of 2.0% methanol was added to test tubes containing 1.0 mL extract solution and incubated at room temperature for 10 min. The blank sample contains 1.0 mL of methanol. Absorbance measurements were performed at 415 nm. The concentration of total flavonoid content in the test samples were expressed as mg quercetin equivalent extracts (mg QE/g-extract) and calculated from the calibration plot using formula (2).

$$y = 0.0152x-0.0648 \quad (R^2 = 0.9804) \qquad (2)$$

Biochemical Analyses. Male albino rats, weighing approximately 150-200 g, were divided into five groups with six animals per group as follows: Group I: control (received water only); Group II: received 0.5% (aq) *C. hederifolium* ethanolic extract of above ground part (Ch AG 0.5); Group III: received 1% (aq) *C. hederifolium* ethanolic extract of above ground part (Ch AG 1.0); Group IV: received 0.5% (aq) *C. hederifolium* ethanolic extract underground part (Ch UG 0.5), and Group V: received 1% (aq) *C. hederifolium* ethanolic extract underground part (Ch UG 1.0). All rats were placed in separate cages and the plant extract was orally administered to the rats for 30 days. The blood samples

were collected from rats before plant extracts administration (on day 0), on the 15th, and 30th days of the experimental period. Blood samples were immersed in ice and centrifuged at 5000 rpm for 5 minutes, the serum was then separated and stored at -20 °C for biochemical analysis. Ethical permission was taken from the Pamukkale University Animal Ethics Committee (No: PAUHDEK-2008/024).

Larvicidal Activity. Houseflies (*M. domestica*) Culture and Larvicidal Assay. Housefly (*M. domestica*), obtained from the strain of the World Health Organization, was used as the 415th generation's larvae which stored in an insectary in the Biology Department, Pamukkale University. The larvae were reared at 16:8 light/dark photoperiod, 50 ± 10% RH, and 26 ±2 ºC. The second-third instar larvae were used for bioassays. Larvicidal activities of plant extracts were investigated by modifying the Çetin *et al.* method [23].

Mosquitos (*Cx. pipiens*) Culture and Larvicidal Assay. Mosquito (*Cx. pipiens*) used in the assays were collected from a pool in August 2019. The larvae were reared at 12:12 light/dark photoperiod, 60 ± 10% RH, and 26 ± 2 ºC in an insectary in the Biology Department, Akdeniz University. The second-third instar larvae were used for bioassays. The larvicidal activity of the extracts were done according to the method of Oz *et al.* [24].

Statistical Analyses. All assays were performed in 3 replicates except biochemical analyses. All values were expressed as mean ± standard error (SE). The results were analyzed using the SPSS (Statistical Package for Social Sciences) statistical software [25]. Significant differences among groups were identified by one-way analysis of variance (ANOVA) with Duncan's multiple range (antioxidant, determination of contents, larvicidal assays) and Tukey tests (biochemical assays), setting $p \leq 0.05$ as the level of significance. $LC_{50(min)}$, LC_{50}, $LC_{50(max)}$, LC_{90}, and x^2 were made by Probit analysis in STATPLUS program [26] in larvicidal activity assays.

RESULTS AND DISCUSSION

It is not possible to determine the plants' antioxidant activities with a single antioxidant activity assay due to the polyphenols substances they contain. It must support each other with more than one antioxidant and quantification experiments.

In vitro Antioxidant Activity and Determination of Contents. DPPH and ABTS free radical scavenging activity assays were calculated as IC_{50} value. The IC_{50} values of the extract concentrations in µg/mL were obtained from the graph drawn from the tested samples. The lower the IC_{50} means, the higher the antioxidant activity [27]. The highest value with the lowest IC_{50} value in the DPPH test was obtained from the ethanol extract of the above ground (0.83 ± 0.00 µg/mL, IC_{50}) and the highest IC_{50} value obtained from the acetone extract of the above ground (2.51 ± 0.01 µg/mL, IC_{50}) (Table 1). There is a statistical difference between all parts, including BHA ($p \leq 0.05$).

Turan and Mammadov [28] found that acetone extract of tubers of *C. alpinum* showed the best antioxidant result with 86.73 ± 0.16% in DPPH assay. It has been also found that the best antioxidant result with 88.25% of the water extract of leaves of *C. mirabile* using DPPH test [29]. Similarly in this study we obtained the best antioxidant activity with the ethanol extract of the above ground of the *C. hederifolium*. The highest value with the lowest IC_{50} value in the ABTS test was obtained from the methanol extract of the underground part (0.16 ± 0.00 µg/mL, IC_{50}) and the highest IC_{50} value obtained from the acetone extract of the above ground (0.28 ± 0.00 µg/mL, IC_{50}) (Table 1). The acetone extract of the leaf part of *C. alpinum* has showed the best free radical scavenging activity with a value of 76.21 ± 0.32% in the ABTS assay [28]. Unlike, in this study, it has been found using ABTS assay that while the acetone extract of the above ground *C. hederifolium* has the lowest free radical scavenging activity, the methanol extract of the underground extract has the highest. These data were similar to Stanojevic *et al.* [30] findings that which were founded the best EC_{50} values of the aqueous extract of *C. purpurascens* tubers as 0.743 ± 0.003 mg/mL using ABTS test.

TABLE 1
Antioxidant activity of *C. hederifolium* extracts

Sample	DPPH (IC_{50}, µg/mL)	ABTS (IC_{50}, µg/mL)
Above ground Part Ethanol	0.83 ± 0.00^a	0.24 ± 0.00^a
Above ground Part Methanol	1.00 ± 0.00^b	0.20 ± 0.00^b
Above ground Part Acetone	2.51 ± 0.01^c	0.28 ± 0.00^c
Underground Part Ethanol	2.41 ± 0.00^d	0.19 ± 0.00^d
Underground Part Methanol	1.20 ± 0.00^e	0.16 ± 0.00^e
Underground Part Acetone	1.61 ± 0.00^f	0.25 ± 0.00^f
BHA	0.03 ± 0.00^g	0.09 ± 0.00^g

abcdefg: Means that do not share a letter in the same line are significantly different from each other ($p \leq 0.05$)

TABLE 2
Determination of total phenolic and flavonoid contents of *C. hederifolium* extracts

Sample	Total Phenolic Content (mg GAE/g-extract)	Total Flavonoid Content (mg QE/g-extract)
Above ground Part Ethanol	11.34 ± 0.04[a]	11.25 ± 0.14[a]
Above ground Part Methanol	13.74 ± 0.10[b]	14.14 ± 0.21[b]
Above ground Part Acetone	9.61 ± 0.06[c]	9.75 ± 0.04[c]
Underground Part Ethanol	4.85 ± 0.15[d]	9.47 ± 0.08[c]
Underground Part Methanol	8.54 ± 0.16[e]	10.64 ± 0.04[d]
Underground Part Acetone	3.95 ± 0.02[f]	8.64 ± 0.06[e]

abcdefg: Means that do not share a letter in the same line are significantly different from each other ($p \leq 0.05$)

Highest phenolic contents were found in the methanol extract of the above ground *C. hederifolium* with a maximum value of 13.74 ± 0.10 mg GAE/g-extract in the total phenolic contents assay (Table 2). There is a statistical difference between all parts, including BHA ($p \leq 0.05$). It has been shown that ethanolic extract of the *C. graecum* leaf has the highest phenolic acid content with a value of 33.73 ± 0.69 µg of PES/mg of extract [31]. Also, petroleum ether extract of the *C. mirabile* leaf has the highest phenolic acid content with the value of 37.36 ± 0.87 µg of PES/mg of extract [29]. Additionally, the highest amount of phenolic substance was found in the above ground *C. alpinum* acetone extract with the value of 8.95 ± 0.87 mg GAE/g [28]. While *C. hederifolium* was observed to have good phenolic contents, the reason why other studies were higher than our study may be due to the differences in the substance used, and extraction method differences. Total flavonoid contents of *C. hederifolium* extracts were determined with aluminum chloride using the spectrophotometric method, and the results varied between 14.14 ± 0.21 - 8.64 ± 0.06 mg QE/g-extract (Table 2). No statistically significant difference was observed in acetone extracts from the above ground, and ethanol extracts from the underground part of *C. hederifolium* ($p > 0.05$), and a statistically significant difference was observed between other parts ($p \leq 0.05$). Turan and Mammadov [28] were seen as the highest amount of flavonoid substance in the acetone extract of the above ground of *C. alpinum* with 92.63 ± 0.45 mg QE/g.

Biochemical Analyses. Values for the biochemical parameters analysed in this study are summarised in Table 3. In order to assess biochemical parameters based on the groups on different measuring days, the values were examined statistically. No difference in CRE values was found among the groups for none of measuring day ($p > 0.05$). On day 0, mean ALT value in Ch UG 1.0 group was significantly higher than those in control, Ch AG 0.5, and Ch UG 0.5 groups ($p \leq 0.05$), whereas mean ALP values in Ch UG 0.5 and Ch UG 1.0 groups were significantly higher than those in control, Ch AG 0.5, and Ch AG 1.0 groups ($p \leq 0.05$). On day 15, mean ALT value in Ch UG 1.0 group was significantly higher than Ch UG 0.5 group ($p \leq 0.05$). Mean ALP values in Ch UG 0.5 and Ch UG 1.0 groups were slightly higher than those in all the groups ($p > 0.05$), but they were found to be significantly higher than only Ch AG 0.5 group ($p \leq 0.05$). On day 30, mean ALT value in Ch AG 1.0 group was significantly higher than those in control, Ch AG 0.5, Ch UG 0.5, and Ch UG 1.0 groups ($p \leq 0.05$). On the same day, mean ALP values in the Ch AG 1.0, Ch UG 0.5, and Ch UG 1.0 groups were significantly higher than those in control and Ch AG 0.5 groups ($p \leq 0.05$). In addition, mean ALP value in Ch AG 1.0 group was higher than that in Ch UG 1.0 group ($p \leq 0.05$).

Temporal changes of biochemical parameters for each group were analysed statistically. Mean CRE value in Ch AG 1.0 group significantly decreased on day 30 compared to day 0 ($p \leq 0$ .05), while mean ALT value in Ch AG 1.0 group showed a significant increase on day 30 in comparison with day 0 and day 15 ($p \leq 0.05$). However, mean ALT value in Ch UG 1.0 group significantly decreased on day 30 compared to day 0 and day 15 ($p \leq 0.05$). Mean ALP value in control group significantly increased on day 15 compared to day 0 ($p \leq 0.05$), and decreased on day 30 compared to day 15 ($p \leq 0.05$). Mean ALP values in Ch UG 0.5 and Ch UG 1.0 groups significantly decreased on day 30 compared to day 0 and day 15 ($p \leq 0.05$), while that in Ch AG 1.0 significantly increased on day 15 compared to day 0 ($p \leq 0.05$), but slightly increased on day 30 compared to day 15, which was statistically insignificant ($p > 0.05$). Mean ALP value in Ch AG 0.5 group displayed a significant increase on day 15 compared to day 0 ($p \leq 0.05$), and showed a significant decrease compared to both day 0 and day 15 ($p \leq 0.05$).

Liver injury can be hepatocellular, cholestatic, or mixed hepatocellular/cholestatic, and the elevation of ALT is associated with the hepatocytes damages which cause the leakage of the ALT from the damaged hepatocytes [32]. Cholestatic injury is originated from the alterations in bile homeostasis and/or bile duct injury and elevated serum ALP level is an indicator for hepatic cholestasis [33]. There was a lot of study about the effects of the plant extracts on liver. Sing *et al*. [34] showed that *Catharanthus*

roseus crude extract decreased the liver enzymes activities in diabetic rats. Researchers have also shown the antihepatotoxic activity of *Rosmarinus tomentosus* [35,36] It has been reported that *Momordica dioica* ethanolic extract has the hepatoprotective action, *in vivo* antioxidant and free radical scavenging activities, and they suggested that possible mechanism of this activity might be due to the presence of flavonoids in the extracts [37]. *C. hederifolium* ethanolic extracts of underground and above ground parts are both contain the flavonoids. Except Ch AG 1.0, all experimental groups did not cause any damages on the liver. As mentioned above, these effects might be due to the flavonoid content of the *C. hederifolium* above ground and underground ethanolic extracts.

In the contrary, medicinal plant can cause hepatotoxicity, or liver damage [38]. For example, green tea widely used all around the world. But it has been concluded that consumption of the high concentrations of green tea extract can cause acute hepatotoxicity in the rat involving in the suppression of major antioxidant enzymes [39,40]. In this study only Ch AG 1.0 group caused the increase in the ALT and ALP values. These elevation of the ALT and ALP levels in the Ch AG 1.0 were indicated the liver damages. Ethanol extract of above ground of *C. hederifolium* has flavonoid content more than the underground part (Table 2). It has also the lowest antioxidant activity (Table 1). Flavonoids have the prooxidant properties, and they can react with organic molecules (like lipids, proteins, and DNA) and induce oxidative damages [41]. When all this is taken into account, it can be suggested that the higher concentration than 1% of ethanol extract of above ground of C. hederifolium can cause hepatotoxicity because of the lowest antioxidant activity and highest flavonoid contents. Also, tannins like phenols are general toxins for many herbivores that significantly reduce the growth and survivors [42]. Additionally, *Cyclamen* extracts contain saponins which are probably toxic and the cytotoxic activities of *Cyclamen* taxa may be due to the this saponin contents [43]. There may be another reason for liver damaging effects of the higher concentration than 1% of above ground ethanol extract of *C. hederifolium* may be related to it's phenolic and saponin contents.

The administration of the *Aloe vera* gel extract to neonatal streptozotocin-induced type-II diabetic rats was decreased serum CRE levels [44]. Also, it has been shown that *Beta vulgaris* extract significantly reduced serum CRE levels of diabetic rats [45]. On the other hand, it has been reported that the aqueous extract of *Vitex doniana* and ethanol/potash extract of the leaf sheath of *Sorghum bicolor* could be toxic to both the liver and kidney [46, 47]. In this study, only Ch AG 1.0 extract was decreased the serum CRE levels from day 0 to day 30. The other extracts did not show any effects on the serum CRE levels and did not cause any kidney damages.

TABLE 3
The biochemical parameters for liver and kidney in the groups.

GROUPS	ALT			ALP			CRE		
	Day 0	Day 15	Day 30	Day 0	Day 15	Day 30	Day 0	Day 15	Day 30
Control	49.67 ± 2.84^{b,x}	54.33 ± 1.41^{ab,x}	56.17 ± 1.38^{b,x}	100.17 ± 15.43^{b,y}	143.83 ± 12.92^{ab,x}	67.50 ± 3.00^{c,y}	0.40 ± 0.02^{x}	0.42 ± 0.02^{x}	0.42 ± 0.02^{x}
Ch AG 0.5	49.67 ± 2.22^{b,x}	50.50 ± 1.34^{ab,x}	48.00 ± 1.81^{b,x}	90.67 ± 1.69^{b,y}	104.50 ± 1.65^{b,x}	82.50 ± 1.48^{c,z}	0.40 ± 0.01^{x}	0.42 ± 0.01^{x}	0.41 ± 0.02^{x}
Ch AG 1.0	53.00 ± 2.22^{ab,y}	52.00 ± 1.34^{ab,y}	72.83 ± 1.81^{a,x}	95.67 ± 1.69^{b,y}	142.33 ± 1.65^{ab,x}	158.67 ± 1.48^{a,x}	0.46 ± 0.01^{x}	0.43 ± 0.01^{x,y}	0.39 ± 0.02^{y}
Ch UG 0.5	46.33 ± 1.58^{b,x}	48.83 ± 1.82^{b,x}	49.50 ± 1.71^{b,x}	173.67 ± 2.76^{a,x}	171.83 ± 7.29^{a,x}	150.33 ± 1.45^{ab,y}	0.43 ± 0.03^{x}	0.40 ± 0.01^{x}	0.40 ± 0.02^{x}
Ch UG 1.0	60.33 ± 1.93^{a,x}	59.33 ± 1.52^{a,x}	51.17 ± 1.25^{b,y}	173.33 ± 1.50^{a,x}	172.83 ± 1.14^{a,x}	129.17 ± 8.21^{b,y}	0.43 ± 0.02^{x}	0.43 ± 0.01^{x}	0.42 ± 0.01^{x}

$^{a, b, c}$: Means that do not share a letter in the same column are significantly different from each other ($p \leq 0.05$). $^{x, y, z}$: Means that do not share a letter in the same line are significantly different from each other ($p \leq 0.05$). Values are represented as mean ± SE; (Ch: *C. hederifolium*; AG: Above ground; UG: Underground; 0.5: 0.5% (aq) ethanolic extract; 1.0: 1% (aq) ethanolic extract) Tukey

TABLE 4
Larvicidal activity of underground and above grounds of *C. hederifolium* against the housefly (*M. domestica*) (1 mg/mL % + S. E.)

	Above ground Part	Underground Part
Negative Control (distilled water)	0.000 ± 0.00^{a}	0.000 ± 0.00^{a}
C. hederiflorum	30.45 ± 2.78^{b}	25.55 ± 2.78^{b}
Positive Control*	100 ± 0.00c	100 ± 0.00c

^{a,b,c}: Means that do not share a letter in the same column are significantly different from each other ($p \leq 0.05$).
* Difluban %48 SC.

Larvicidal Activity Against Housefly (*M. domestica*) and Mosquitos (*Cx. pipiens*). The larvicidal activity of *C. hederifolium* against *M. domestica* was investigated, and the results are shown in Table 4. In the assay, only water extract was used against the 2nd and 3rd instar larvae. The period, in which adult emergence was observed in 25 larvae in the control group, was determined as the duration of the assay and the best result was provided in the positive control [Difluban %48 SC, active ingredient: Diflubenzuron (CAS No: 35367-38-5)], and no adult emergence was observed at the end of the period (100 ± 0.0% died rate). Although there is not much difference between the above ground and the underground part of *C. hederifolium* extracts, the above ground part of *C. hederifolium* extract has found to be more active, with 30.45 ± 2.78%. In the assay results, a statistically significant difference was found between the controls and underground and above ground parts extracts of *C. hederifolium* ($p \leq 0.05$). In the study by Morey and Khandagle [48], the larvicidal effect of the essential oils of *Mentha piperita* L. (Family: Lamiaceae), *Zingiber officinale* Roscoe (Family: Zingiberaceae), *Emblica officinalis* Gaertn (Family: Phyllanthaceae), and *Cinnamomum verum* J. Presl (Family: Lauraceae) against *M. domestica* was examined, and 104 ± 0.31 (ppm, LC_{50}) was observed in the highest larvicidal activity with *M. piperita*. Compared to our study, it was seen that better results were obtained, and *C. hederifolium* was not active to *M. domestica*.

Results of larvicidal activity of *C. hederifolium* extract against different instar larvae of *Cx. pipiens* are shown in Table 5. In the assay, only water extract was used against the 2nd and 3rd instar larvae. The best result was positive control (Mozkill 120 SC, active ingredient: Spinosad, CAS No: 168316-95-8), and 100% result was observed within 1 hour. After 72 hours of exposure, the above ground part of *C. hederifolium* extract showed the most toxic effect, with the value of 83.33 ± 0.00% (0.271 mg/mL, LC_{50}). Concentration and time of exposure were found to be effective in increasing larvicidal activity. Statistically, there is a difference in all reading times in terms of duration at 1 mg/mL in the above ground part of *C. hederifolium* extract ($p \leq 0.05$), and there was no statistical difference between 48 and 72 hours of exposure at 1 mg/mL in the underground part of *C. hederifolium* extract ($p > 0.05$). In Oz *et al.* [24] study with *Cyclamen* species (*C. mirabile* and *C. alpinum*), they found best result with the value of 86.6 ± 2.6% in 3-4 instar larvae after 72 hours of exposure at the concentration of 1 mg/mL of the underground part extracts. Similarly, Turan and Mammadov [28] found the best result with the value of 100 ± 0.0% (0.151 mg/mL, LC_{50}) in the *C. alpinum* underground extract after 72 hours of exposure at the concentration of 1 mg/mL.

TABLE 5
Average mortality rates (%) of the *C. hederifolium* concentrations at the time of exposure to mosquitos (*Cx. pipiens*) 1 mg/mL % ± S.E.

	Above ground Part 24 h later	Above ground Part 48 h later	Above ground Part 72 h later	Underground Part 24 h later	Underground Part 48 h later	Underground Part 72 h later
Negative Control (distilled water)	$0 \pm 0.0^{A,a}$	$2.78 \pm 2.78^{A,a}$	$2.78 \pm 2.78^{A,a}$	$0 \pm 0.0^{A,a}$	$2.78 \pm 2.78^{A,a}$	$5.56 \pm 2.78^{A,a}$
0.1 g/mL	$5.56 \pm 2.78^{A,ab}$	$3.89 \pm 2.78^{B,b}$	$25.00 \pm 4.81^{C,b}$	$2.78 \pm 2.78^{A,ac}$	$5.56 \pm 2.78^{A,a}$	$8.33 \pm 4.81^{A,a}$
0.25 mg/mL	$22.22 \pm 5.56^{A,b}$	$0.56 \pm 2.78^{B,c}$	$44.44 \pm 2.78^{B,b}$	$8.33 \pm 4.81^{A,a}$	$30.56 \pm 5.56^{B,b}$	$41.67 \pm 4.81^{B,b}$
0.5 mg/mL	$47.22 \pm 2.78^{A,c}$	$5.56 \pm 2.78^{B,cd}$	$69.44 \pm 2.78^{C,c}$	$27.78 \pm 2.78^{A,b}$	$72.22 \pm 2.78^{B,c}$	$77.78 \pm 2.78^{B,c}$
1 mg/mL	$50.00 \pm 4.81^{A,c}$	$9.44 \pm 2.78^{B,d}$	$83.33 \pm 0.00^{C,c}$	$33.33 \pm 4.81^{A,b}$	$72.22 \pm 5.56^{B,c}$	$80.56 \pm 2.78^{B,c}$
Positive Control*	$100 \pm 0.0^{A,d}$	$00 \pm 0.0^{A,e}$	$100 \pm 0.0^{A,d}$	$100 \pm 0.0^{A,c}$	$100 \pm 0.0^{A,d}$	$100 \pm 0.0^{A,d}$
LC_{50} (min) (mg/mL)	0.287	0.360	0.222	1.141	0.177	0.048
LC_{50} (mg/mL)	0.782	0.475	0.271	1.608	0.413	0.327
LC_{50} (max) (mg/mL)	2.137	0.586	0.325	2.910	0.968	1.465
LC_{90} (mg/mL)	4.906	2.768	1.582	10.023	1.510	1.167
x^2	3.973	0.406	0.434	2.773	5.815	3.394

^{ABC}: Means that do not share upper cases in the same line are significantly different from each other ($p \leq 0.05$). ^{abcde}: Means that do not share a letter in the same column are significantly different from each other ($p \leq 0.05$). *Mozkill 120 SC

In the study by Aydın and Mammadov [49], the best result was found in the bulb extract of *Hyacinthella lineata* with the value of 64.327 µg/mL (LC_{50}) after 72 hours of exposure. Although we found in this study that the above ground part of *C. hederifolium* extract showed the most toxic effects on different instar larvae of *Cx. pipiens,* because of the liver damaging effects of this extract and according to the mentioned data, it will be suitable to use underground *C. hederifolium* extract as larvicide. Already, *C. hederifolium* tubers are used as a traditional insecticide by tubers pounding and putting them into a certain amount of water, and then infusing the filtered water to tobacco seedlings.

CONCLUSION

Based on the findings of this study, it can be concluded that no used dose and type of extracts have a negative effect on the kidneys. Considering the ALT and ALP measurements, the increase in the values in Ch AG 1.0 group over time indicates liver damage. Above ground part of *C. hederifolium* extract (1%) showed the best larvicidal activity against the housefly (*M. domestica*) and mosquito (*Cx. pipiens*), but it may not be suitable to be used as a larvicide due to its possible hepatotoxic effect. It seems that underground part of *C. hederifolium* extract (1%) reduces liver enzyme levels, and it has increased larvicidal effect over time. Therefore, it can be suggested that underground part of *C. hederifolium* extract (1%) can be used as a larvicide to protect not only the environment but also the organisms. However, further studies are needed to isolate and identify the active components of these extracts and to understand their mechanisms of action.

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