INTRODUCTION

The *Delphinium* L. genus (Ranunculaceae), with almost 300 species worldwide, consists of 29 species in Turkey (17 endemic to Anatolia). Their usages have a long history of medicinal use. Dioscorides mentioned their use against lice and scorpions. The powders of *D. staphisagria* L. and *D. peregrinum* L. were used against lice by the British army in the battle of Waterloo. The dried and mature seeds of *D. staphisagria*, known as "kokarot, kokarotu, müzüdek, mevezek and mevzek" (Baytop 1999) were used as emetics, purgatives and sedatives (in the treatment of rabies, tetanus, and epilepsy) in traditional Turkish medicine. The usage was left aside due to their high toxicity. Despite their toxicity, their external applications (as ointment, powder form or infusion (1-

The cytotoxicity and insecticidal activity of extracts from *Delphinium formosum* Boiss. & Huet

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ABSTRACT

*Delphinium* species are well-known toxic plants with diterpenoid alkaloid contents. There has been no previous investigation on the cytotoxicity of *Delphinium formosum*. The extracts of the different parts of *D. formosum*, an endemic species in Turkey, were investigated for their cytotoxic activity against the human liver carcinoma cell line (HepG2) and primary human umbilical vein endothelial cells (HUVEC). The cytotoxic effects of twelve extracts and subfractions were determined against HepG2 cells using the MTT assay. The only active extract was applied to the HUVEC as a model for healthy cells. Only one of the alkaloid-containing extracts from the aerial parts was toxic (IC50=244.9 µg/mL against HepG2 and 144.4 µg/mL against HUVEC), while the root extracts were inactive. The results were improbable although it is predicted secondary metabolites, such as diterpene alkaloids (methyllycaconitine, browniine, lycoctonine, avardharidine, antranoyllycoctonine, delsemine A/B and lycoctonine). Based on previous studies in the literature, the cytotoxic plants were also expected to exhibit insecticidal activity. Therefore, the cytotoxic extract of *D. formosum* was examined for its adulticidal and larvicidal activity against the yellow fever, dengue fever and the Zika virus vector *Aedes aegypti*. Keywords: *Delphinium formosum*, cytotoxic activity, HepG2, HUVEC, natural insecticidal, *Aedes aegypti*

INTRODUCTION

The *Delphinium* L. genus (Ranunculaceae), with almost 300 species worldwide, consists of 29 species in Turkey (17 endemic to Anatolia). Their usages have a long history of medicinal use. Dioscorides mentioned their use against lice and scorpions. The powders of *D. staphisagria* L. and *D. peregrinum* L. were used against lice by the British army in the battle of Waterloo. The dried and mature seeds of *D. staphisagria*, known as “kokarot, korkarotu, muzudek, mevezek and mevezek”, (Baytop 1999) were used as emetics, purgatives and sedatives (in the treatment of rabies, tetanus, and epilepsy) in traditional Turkish medicine. The usage was left aside due to their high toxicity. Despite their toxicity, their external applications (as ointment, powder form or infusion (1-

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Vector-borne diseases cause epidemics, leading to serious human health problems. *Aedes aegypti* (Culicidae), one of these vectors, transmits viruses like Dengue, Yellow fever, Zika, and Chikungunya. According to WHO, yellow fever is an acute viral haemorrhagic disease transmitted by infected mosquitoes, and Dengue is a mosquito-borne viral infection, which causes a flu-like illness, and evolves on occasion into lethal complications. WHO also emphasized that, the symptoms of the infection of Zika virus, transmitted primarily by *Aedes* mosquitoes, are skin rash, mild fever, muscle and joint pain, headache, conjunctivitis or malaise, and is also a cause of Guillain-Barré syndrome and microcephaly (Masi et al. 2017; WHO February 2018; WHO May 2018; WHO April 2019). Many interventions are conducted to prevent these diseases, and one of them is integrated mosquito control, including the implementation of personal protection, destruction and reduction of its habitats, and insecticidal treatment regimens against adult and larval mosquitoes for the reduction of the virus spread. Reducing the amount of synthetic insecticides or pesticides applied, is preferred because of their undesirable and toxic effects. Frequent chemical interventions can also cause the development of insecticide resistance (Tabanca et al. 2013a; Masi et al. 2016). Therefore, an increasing number of plant-based extracts, essential oils or phytochemicals are being investigated with the aim of determining an effective agent against *A. Aegypti* (Kamaraj et al. 2010; Pitarokili et al. 2011; Maheswaran and Ignacimuthu 2012; Liu et al. 2012; Kumar et al. 2012; Tabanca et al. 2013a, b; Reegan et al. 2015; Cantrell et al. 2016; Masi et al. 2016; Dias et al. 2017; Carroll et al. 2017; Chantawee and Soonwera 2018; Stappen et al. 2018; Tabanca et al. 2018, 2016a, 2016b).

The alkaloids, one of the most remarkable groups of natural products in these plants, have a wide variety of biological activities. The structures of over a hundred alkaloids have been identified; many of them have elicited an expected anticaner activity (Lu et al. 2012). In addition to the studies on cytotoxic and anticaner effects (Liu et al. 2017; Nugroho et al. 2015; Chanakul et al. 2011), several investigations into insecticidal activities have been published (Bandara et al. 2000; Garcez et al. 2009; Liu et al. 2012; Masi et al. 2017). The diterpene and nor-diterpene alkaloids have also shown insecticidal activity (Ulubelum et al. 2001; Kukel and Jennings 1994). Also based on previous studies in literature, the cytotoxicity and insecticidal activities are related to each other, and diterpene alkaloids can be protective agents for parasit control strategies (Gonzalez-Coloma et al. 2004; Reina and Gonzalez-Coloma 2007).

This preliminary study was presented to assess the cytotoxic and insecticidal potentials of extracts, obtained from *Delphinium formosum* an endemic species in Trabzon (Turkey), which has a role in traditional medicine and causes serious poisoning. The different extracts of its aerial parts, roots, and flowers were investigated for their cytotoxic activity against the human liver carcinoma cell line (HepG2) and primary human umbilical vein endothelial cells (HUVEC). The mosquitocidal activity of the cytotoxic extract against *Ae. aegypti* was also determined for the possibility to use its lethal potential as an insect repellent.

**MATERIALS AND METHODS**

**Plant material**

*Delphinium formosum* was collected from Köprübaşı-Trabzon and Macka-Trabzon, in August 2011 and July 2012. The voucher specimen has been deposited in the Herbarium of the Faculty of Pharmacy, Istanbul University (ISTE 102747; 98086).

**Extraction procedure**

Dried and powdered roots, collected in the first year, were percolated with EtOH in four portions (10 L, 3 L, 2 L, 5 L) and each portion was evaporated to dryness at 40°C in vacuo (DW1, DW2, DW3 and DW4). The residues of the portions (DW1 and DW2) were acidified to pH1 by 200-300 mL 0.5 N H$_2$SO$_4$ and extracted with CHCl$_3$. These CHCl$_3$ extracts were evaporated to dryness (DW1A1K and DW2A1K). The acid solutions were basified with 5% NaOH to pH10 and extracted a second time with CHCl$_3$. The extracts were evaporated to dryness, and named as DW1A2K and DW2A2K (Figure 1).

Dried and powdered aerial parts, flowers and roots, collected next year, were percolated with EtOH in one portion and evaporated to dryness at 40°C in vacuo. The same process was applied. Two different chloroform extracts of each part (HA1K, HA2K, FA1K, FA2K, 2DWA1K, and 2DWA2K) were acquired (Figure 2).

**Biological Assays**

**Cytotoxicity Assays**

**Cytotoxicity test, cell lines, culture conditions and treatments**

Roswell Park Memorial Institute Medium 1640 (without glutamine; RPMI-1640) with glutamine, 10% FCS and penicillin / streptomycin mixture was used for the human liver carcinoma cells (HepG2). Primary human umbilical vein endothelial cells (HUVEC) were isolated from umbilical cords by digestion with 0.01% collagenase A solution (Roche) and grown in Endothelo-

![Figure 1. Representative scheme of extraction procedure of the roots, collected in the first year.](image-url)
Cytotoxicity studies

For MTT tests, the cells were then incubated with 150 µL MTT (0.5 mg/mL in medium) solution for 3 h. After the removal of the MTT solutions, the formed formazan crystals were solubilized in 80 µL of DMSO and then, the absorbance was measured at 550 nm and at 690 nm (as control wavelength) using a microplate reader. The absorbance of the test samples was corrected for background absorbance using an equal volume of DMSO as a solvent control. The percentage of cell viability was calculated with respect to solvent control as follows:

\[
\% \text{ Cell viability} = \frac{\text{Abs}_{\text{Compound}}}{\text{Abs}_{\text{Solvent Control}}} \times 100
\]

The results were expressed as cell death (%) compared to the negative control.

Insecticidal Activity

Mosquito Colony

Aedes aegypti used for testing were pesticide susceptible, and provided by the CMAVE insectary. The "Orlando1952" strain was collected near Orlando, Florida, USA in 1952, and has been in continuous laboratory colony for 64 years. Rearing procedures are standardized and have been described previously (Tabanca et al. 2016b).

Larvicidal Activity

Larvicidal activity testing was performed essentially as described previously (Pridgeon et al. 2008), but the assay was modified for 96-well plates to conserve limited amount of test samples by the use of smaller volumes (Masi et al. 2017). The HA1K extract was diluted in dimethyl sulfoxide (DMSO) to make 100 mg/mL. Mortality was determined in the larval assays at four different concentrations (1.0, 0.5, 0.25, and 0.1 μg/μL) in a final volume of 200 μL of larval rearing media. For each assay, a positive control of permethrin stock and a negative control of ethanol or DMSO was included. Assays were repeated at least three times on separate days using different hatches of eggs.

Adulticidal Activity

The toxicity of HA1K extract was tested in assays against adult Aedes aegypti using cohorts of 3-6-day post-emergence females as described previously (Pridgeon et al. 2008). Mosquitoes were cold anesthetized on ice, and groups of 10 females sorted into individual plastic cups. An application of 0.5 μL of the appropriate dilution of the test chemical was made by repeater pipettor (Hamilton PB600) with a 25 μL blunt tip glass syringe (Hamilton 7100 series) to at least twenty females at each dose. Permethrin mixture of 46.1% cis and 53.2% trans isomers (Chemservice, West Chester, PA) was used as a positive control, and acetone was used as negative control. After treatment, the mosquitoes were kept in plastic cups at 24-26°C and 80% humidity, and supplied with 10% sucrose in water for 24 h prior to recording mortality.

RESULT AND DISCUSSION

Due to the diterpenoid alkaloid content of Delphinium species, we expected distinct cytotoxic actions when testing their effect on HepG2 cells. Only one extract (HA1K) was active among them, in the studied concentration ranges. The same extract was also examined against the HUVEC cells. The IC_{50} values of HA1K against two cell types (244.9 μg/mL for HepG2 and 144.4 μg/mL for HUVEC) were similar.
The MTT assay demonstrated the effect of the extracts on the % cell viability of HepG2 and HUVEC cell lines as shown in Figure 3.

The HA1K extract, found cytotoxic against both cell lines, was further investigated for its insecticidal activity (Table 1). The extract was evaluated at the dose of 5 mg/mosquito and had 97% mortality against adult A. aegypti. The positive control permethrin at 6.33 pg/mL resulted in 53±11% mortality, while the negative control acetone resulted in 0% mortality. In larval activity, the HA1K extract showed 80% mortality at the highest dose of 1 mg/mL against 1st instar A. aegypti; however, the mortality tittered off quickly at the lower doses. Negative control mortality in larvicidal assays was 0% for DMSO, and positive control permethrin resulted in 100% at the 47.4 pg/mL. Since HA1K extract produced 97% mortality against adult A. aegypti, isolation of the active compound/s through bioassay-guided fractionation and its/their characterization may be promising.

This is the first investigation on the cytotoxicity and insecticidal activity of D. formosum. On the other hand, several studies on the cytotoxic effect of the isolated compounds from other species exist in the literature. De Inés et al. evaluated 43 norditerpenoid alkaloids from Aconitum, Delphinium and Consolida species for their cytotoxic effects on the tumor cell lines SkMel28 (human malignant melanoma), HeLa (human cervical adenocarcinoma), SkMe125 (human melanoma), CT26 (murine colon adenocarcinoma), and SW480 (human colon adenocarcinoma) and the non-tumor cell line CHO (Chinese hamster ovary cells). Browniine, ajadelphinine, 8-O-methylcolumbianine, dehydrotakomasine, lycoctonine, 14-deacetylajacine, pubescenine, 14-deacetylpubescenine, 1,14-diacetyldiordacetaparine, 18-O-demethylpubescenine, delphatine and neoline showed selective cytotoxicity. The most active compound was a gadesine-type norditerpene alkaloid, dehydrotakomasine. Its IC50 values were detected 0.40 μg/mL against HeLa cells and 6.25 μg/mL against CT26, SW480 and SkMe125 cells. Lycoctonine was found active against CT26 and SW480 (IC50=50 μg/mL), while methyllycaconitine showed an activity against CHO, CT26, SW480 and HeLa (IC50=12.50 μg/mL, 12.50 μg/mL, 50 μg/mL, 50 μg/mL, respectively). All IC50 values of browniine were more than 100 μg/mL against all cell lines, but an effect was seen by browniine against CT26 and SW480 cells (De Ines et al. 2006). In another study, from Aconitum vulparia isolated compounds (vulparine, finetia-dine, anthranoyllycoctonine) exhibited a cell growth inhibitory activity against cervix adenocarcinoma (HeLa) and breast adenocarcinoma (MCF-7) cell lines. Finetia-dine was the most cytotoxic compound at 30 μg/mL with the 39.48±3.42% on MCF-7 cell line and 25.59±1.87 % inhibition on HeLa cell line, while septentriodine and anthranoyllycoctonine also showed tumour cell inhibitory activity. Septentriodine showed an activity (34.50±3.72 % inhibition) against MCF-7 cells only at the concentration of 30 μg/mL (Csupor et al. 2007).

In a study on Delphinium alkaloids to determine the inhibition of α-bungarotoxin binding to insect and rat neural membranes, 17 Delphinium alkaloids were tested. Among them, glaudelsine showed the highest inhibition on the insect binding site even 10x more potent than methyllycaconitine, which was known as the most potent alkaloid until that date (Kukel and Jennings 1994). In another study, 29 diterpene and norditerpene alkaloids from Delphinium, Consolida and Aconitum species were investigated against Tribolium castenum (Herbst.) to evaluate the repellent effects. Twenty-one alkaloids exhibited promising insect repellent activity (Ulubelen et al. 2001). Hetisine, a diterpen alkaloid, was found to have the most active repellency (59.12%). The compounds, delsemin-B, lycoctonine and browniine, showed also an activity against Tribolium castenum (Ulubelen et al. 2001).

In a previous study from the Black Sea area (Trabzon) in Turkey, D. formosum was collected and, delsemine A/B, 14-demethyl-jacine (N-acetyldelactine), lycoctonine, anthranoyllycocto-nine, delcosine and delectine were identified from the aerial parts (Mericli et al. 1996). Additionally, lycoctonine and delsemine were isolated from D. formosum roots, collected in Trabzon, in a previous study (Tanker and Ozden 1975). Durust et al. (1999) also isolated some phenolic acids such as p-coumaric, p-hydroxy benzoic, caffic, protocatechic and vanillic acids from the flowers of D. formosum, collected in same region, with four different methods. In our recent study, we reinvestigated norditerpenoid alkaloids from the D. formosum roots, and methyllycaconitine, browniine, lycoctonine, avardharidine, antranoyllycoctonine and delsemine A/B were isolated (Sen-Utsukarci et al. 2018).

All of the aforementioned studies on the cytotoxic effects of diterpenoids and norditerpenoid alkaloids have a contribution on the cytotoxicity. The difference of the cytotoxicity of D. formosum (aerial parts and roots) can be explained with the diversity of secondary metabolites in the root-extracts. In light of it all, it gives the impression that the extracts may contain different percentages of the active substances.

![Figure 3](image-url). The % cell viability values of the HA1K against HepG2 and HUVEC cell lines.

**Table 1. The mortalities of the HA1K against adult female mosquito Ae. aegypti and 1st instar Ae. aegypti larvae**

| Adult female mosquito Aedes aegypti | 1st instar Aedes aegypti larvae (% mortality) |
|-----------------------------------|---------------------------------------------|
| 5 µg/mosquito (% mortality)       | 1 µg/µL 0.5 µg/µL 0.25 µg/µL 0.1 µg/µL          |
| 96.7±5.8                         | 80 53.3±23.1 13.3±11.5 0                   |

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