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Composition of the essential oil and aqueous leachate from *Heracleum mantegazzianum* seeds and their larvicidal activity against an invasive mosquito *Aedes japonicus*

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ABSTRACT

This study evaluated the chemical composition of essential oil and aqueous leachate from seeds of the invasive neophyte *Heracleum mantegazzianum* and tested their larvicidal activity against the invasive mosquito *Aedes japonicus*. Octyl acetate (58.65 %), followed by hexyl 2-methylbutyrate (10.61 %) and hexyl butyrate (9.37 %) dominated the essential oil. Therefore, the larvicidal activity of octyl acetate was tested as well. The 3-hydroxybenzoic acid, syringic acid, and chlorogenic acid were dominant polyphenols in the aqueous leachate. The LC_{50} and LC_{90} values of essential oil were 0.067 mg/mL (0.050-0.088) and 0.179 mg/mL (0.135-0.239), those of octyl acetate were as follows: LC_{50} 0.052 mg/mL (0.041-0.065), LC_{90} 0.103 mg/mL (0.082-0.130). The larvicidal effect of the aqueous leachate was expressively lower; values of $_{LC50}$ and $_{LC90}$ were 9.235 mg/mL (7.144-11.94) and 32.88 mg/mL (25.44-42.50) respectively. Essential oil, octyl acetate, and aqueous leachate caused some mortality in non-target organisms, too; however, they were significantly lower than those of Asian bush mosquito larvae.

Key-words: Aqueous leachate, essential oil, giant hogweed, octyl acetate, larvicidal activity, Asian bush mosquito.

INTRODUCTION

Thousands of plants produce biologically active compounds, including essential oils (EOs), which can be applied as natural mosquitocides (24). Compared to conventional synthetic insecticides, EOs are recognized as non-toxic to humans, have low environmental persistence, and do not accumulate along the food chains (18). The invasive neophytes were confirmed to produce a spectrum of biologically active compounds, making them more successful in the new habitats (9). These compounds, including those mosquitocidal, are undoubtedly worth studying for their biological activity. Moreover, plant invaders typically produce large amounts of aboveground biomass since the necessity of its removal is undoubtedly, and even compulsory, according to actual legislation. The use of harvested

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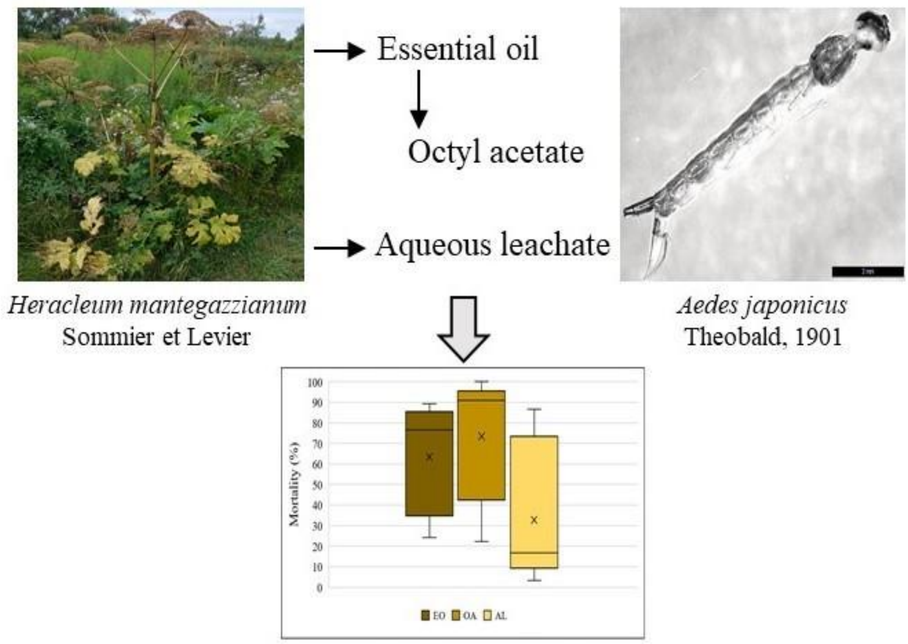
biomass for EOs extraction, and consistent EOs practical application should be a part of the balanced and optimized neophytes management (5).

Giant Hogweed *Heracleum mantegazzianum* Sommier et Levier (Apiales: Apiaceae) originated from the Caucasus (Asia) and was introduced into Europe as an ornamental plant at the beginning of the 19th century (4). Nowadays, Giant Hogweed is found to be one of the most dangerous invasive neophytes throughout Europe. These plants produce many chemical compounds, including furanocoumarins, and volatiles as essential oil present the second class of abundant compounds in the *Heracleum* genus (20,31,35).



Graphical abstract
Testing of the Giant Hogweed biologically active substances for its larvicidal activity

Asian bush mosquito *Aedes japonicus* Theobald, 1901 (Diptera: Culicidae) is a container-dwelling and container-breeding species colonizing natural and man-made breeding sites. Species originated from Japan and Korea, however, in recent decades, it rapidly expanded its range and its occurrence has been confirmed in Central Europe and Northern America. Recently, *Ae. japonicus* is considered one of the most expansive mosquito species in the world (21). The medical importance of *Ae. japonicus*, especially under the climate changes, is undoubtedly a growing concern: the species was proven to be a vector of several flaviviruses, including viruses of Japanese Encephalitis, West Nile virus, and Saint Louis encephalitis virus, as well as arboviruses such as La Crosse virus, Chikungunya virus, dengue and even Zika under the laboratory conditions. Thus, *Ae. japonicus* could potentially serve as a bridge vector for zoonotic arboviruses. Except for, species is a competent vector for even filarial nematodes. Analyses of its meal suggest, that it can serve as a bridge vector of pathogens from avian hosts to humans (10,14,28,30). The best way to their control is to prevent transmission by reducing vector populations. However, facing up the vector's resistance to insecticides, eco-friendly programs should be applied. Despite the concerns, plant-based biological control of *Ae. japonicus* is just significantly less studied.

In this study, we hypothesized, that *H. mantegazzianum* seeds essential oil and its main constituent and aqueous leachate have larvicidal activity against *Ae. japonicus*. We hypothesized that the aqueous leachate effect would be expressively lower than those of EO and OA. Our study aims at initial testing of the Giant Hogweed seeds essential oil (EO), its main compound octyl acetate (OA), and aqueous leachate (AL) larvicidal activity against invasive Asian bush mosquitoes.

MATERIALS AND METHODS

I. Plant Material Collection

Inflorescences of the Giant Hogweed (*Heracleum mantegazzianum* Sommier et Levier) were collected from separate, fully matured maternal plants in 2020. The sampling site was localized within the cadastre of Lekárovce village, Eastern Slovakia (GPS 48°36'16.8500557"N, 22°9'24.3084526"E). Inflorescences were left freely air-dried in the dark for 14 days. After drying out, the seeds were manually separated from the inflorescences. Seeds are large, flat oval, on average 4–18 mm long and 2–10 mm wide, tan, with brown lines. Whole unmilled seeds, including testa, were used for the essential oil extraction and aqueous leachate preparation.

II. Essential Oil Extraction and Chemical Composition Analysis

Essential oil (EO) from the dried seeds of *H. mantegazzianum* was extracted by hydro-distillation in a Clevenger-type apparatus (Kavaliergalss, Sázava, Czech Rep.) for 3 h. The composition of extracted EO was analyzed using GC-MS-FID-gas chromatography-mass spectrometry combined with a flame ionization detector, performed on a Trace GC Ultra coupled with a DSQII mass spectrometer (Thermo Electron, Waltham, MA, USA). A simultaneous GC-FID and MS analysis was performed using an MS-FID splitter (SGE Analytical Science, Ringwood Victoria, Australia). The mass range was 33–550 amu, with an ion source heating of 200 °C and an ionization energy of 70 eV. One microliter of EO solution (80 % v/v) diluted in pentane: diethyl ether was injected in split mode at split ratios (50:1). Operating conditions for capillary column Rtx^{-1} MS (60 m x 0.25 mm i.d., film thickness 0.25 μm), and temperature program: 50 (3 min)–300 °C (30 min) at 4 °C/min. Injector and detector temperatures were 280 °C and 300 °C, respectively. The carrier gas was helium (constant pressure: 300 kPa). There were analyzed each sample twice, the final presented composition consists of the means from these two measurements.

III. Aqueous Leachate Preparation and Chemical Composition Analysis

Dried seeds of *H. mantegazzianum* were immersed in distilled water (20 g/100 mL) and incubated at 60 ºC for 1 h. Leachate was filtered through a Buchner funnel with Whatman No. 1 filter paper and centrifuged at 6 000 rpm (revolutions per min) for 30 minutes. The content of the dry biomass in mg/mL was determined with the purpose of LC_{50} (concentration of a substance that is lethal to 50 % of the organisms in a toxicity test) and LC_{90} (concentration of a substance that is lethal to 90 % of the organisms in a toxicity test) determination). Leachate was used immediately after the preparation.

Selected polyphenols were determined in aqueous leachate prepared as mentioned above. The obtained leachates were analyzed by Agilent 1200 series HPLC-DAD (High-performance liquid chromatography with photodiode-array detection) system (Agilent Technologies, Santa Clara, California, USA). Acetonitrile, water, and formic acid, all HPLC-grade, were purchased from Lach-Ner, s.r.o. (Neratovice, Czech Republic). Standards of selected polyphenols were obtained from Merck KGaA (Darmstadt, Germany). The separation was performed on a Zorbax SB-C18 column (4.6 mm × 150 mm, 5 µm particle size; Agilent Technologies, Santa Clara, California, USA) that was kept at 30 °C throughout the run. The mobile phase comprised acetonitrile (A) and 0.1 % formic acid (B). The following gradient program was employed: 0 min 5 % A; 15 min 60 % A; 20 min 100 % A; 20 – 30 min 100 % A. Before further analysis, the column was equilibrated using the mobile phase containing 5 % A for 10 min. The flow rate was set to 0.7 mL/min, and the injection volume was 5 µL. The analytes were detected at 260, 280, and 360 nm. The identification of target compounds was based on a comparison of their retention times with those of authentic standards and the method of standard addition. Quantitative analysis was performed by the external standard method. Standard calibration solutions were prepared in a concentration range of 0.5–25 µg/mL and triplicate injections were made for each calibration level. Good linearity with a correlation coefficient of r2 > 0.999 for all compounds was achieved in the investigated range. The LOD (s/n=3) - limit of detections was in the range of 0.040 – 0.100 µg/mL for all analyzed compounds. The LOQ (s/n=10) - limit of quantification was in the range of 0.133 – 0.333 µg/mL for all studied compounds. The repeatability of the method (characterized by a relative standard deviation (RSD, %) was RSD < 1 % for all analyzed compounds. The content of target compounds was expressed in µg per g of the dry biomass ± standard deviation (n=2).

III. Larvicide Bioassay

The fourth-instars larvae of the Asian bush mosquito *Aedes japonicus* Theobald, 1901 were used for the bioassay. Larvae were collected from the rainwater barrels at a

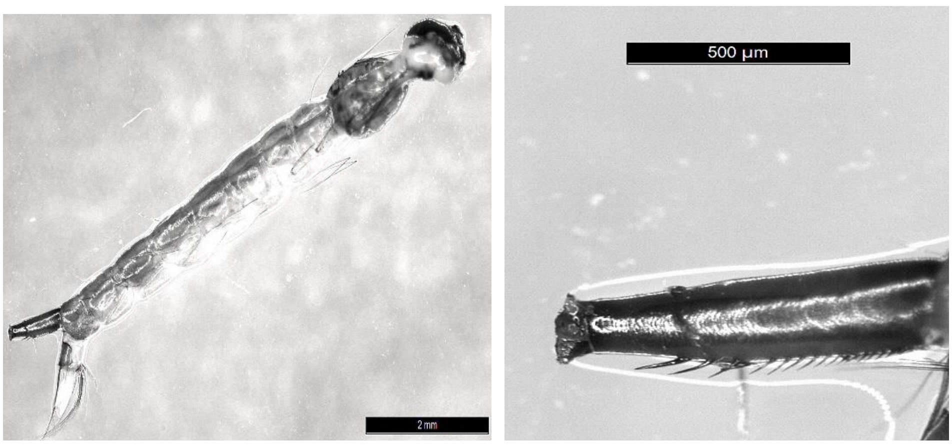


Figure 1. Larvae of Asian bush mosquito and the detail of the siphon.

private garden in Ruská Nová Ves village, Eastern Slovakia (GPS 48°58'14.8348726"N, 21°19'37.3361206"E) and identified (6,12,25). The voucher specimen is kept at the Department of Ecology, FHaNS University of Prešov, Slovakia. Larvae were kept in the rainwater from the barrels in a refrigerator at an average temperature of 4.0 °C till the experiment.

The larvicidal activity was assessed according to the standard methodology suggested by the World Health Organization (38), with slight modification (8). Required aliquots of EO were diluted in the 2 % aqueous dimethylsulfoxide (DMSO) to obtain concentrations range of 0.035, 0.060, 0.085, 0.110, and 0.135 mg/mL (29). In each experiment, ten fourth-instars larvae of Asian bush mosquito were added to the 20.0 mL of EO solution. In each test, 3-experiments were undertaken for each concentration. Three independent trials were run completely (total n=90 larvae). 2 % DMSO water solution was used as a negative control. Experiments were incubated at 25 ± 2 °C. A mortality count was conducted 24 h after the experiment was performed. Larvae were considered dead when they did not spontaneously move, even if subjected to mechanical stimulation with a gentle brush.

As the main compound of *H. mantegazzianum* essential oil octyl acetate (OA) was detected, we subjected OA to identical larvicidal testing as EO. An analytical standard with purity ≥ 98.5 % (purchased from Sigma-Aldrich) was used for bioassay.

The aqueous leachate was diluted to 12.5, 25, 50, and 75 % (v/v) using distilled water, and pure, non-diluted 100 % leachate was used for the experiment too. As a control, distilled water was used. Two separated aqueous leachates were prepared and tested for toxicity, and the contents of the dry biomass responded 1.5, 1.8, 2.9, 3.7, 5.8, 7.3, 8.8, 11, 12, and 15 mg/mL.

As non-target organisms, larvae of two non-biting mosquitos were used: "blood worms" *Chironomus aprilinus* Meigen 1830 (Diptera: Chironomidae) and "glass worms" *Chaoborus* spp. (Diptera: Chaoboridae). Both are commercially available, harmless representatives of the water invertebrates and were exposed to identical tests with *H. mantegazzianum* EO, OA, and aqueous leachate as *Ae. japonicus*.

IV. Data Analysis

The mortality obtained from the larvicidal bioassay of *Ae. japonicus*, *Chi. aprilinus* and *Chaoborus* spp. was corrected using Abbott's formula (13). Larvicidal data were subjected to Finney's probit analysis for determining the LC_{50}, LC_{90}, and 95 % confidence intervals of upper/lower confidence limit (UCL/LCL) and Chi test ($\chi 2$) results (22).

One-way ANOVA was undertaken to distinguish if there are significant differences between model organisms in the larvae mortality. A level of significance $p \leq 0.05$ was taken as significant. Simple linear regression analysis was used to assess the relationship between EO/OA/aqueous leachate concentration and larvae mortality. Statistical analyses were performed in the statistical program PAST Version 4.03 (16).

RESULTS AND DISCUSSION

Essential Oil and Aqueous Leachate Composition

The hydrodistillation of the *H. mantegazzianum* dried seeds yielded 4.9±0.14 % of pale yellow EO with a strong floral odour GC-MS analysis allowed to identify of 52 components in the EO, with a representation of 99.73 % (Table 1). Seven compounds had representation ≥1 % and together with 92.68 %, since octyl acetate was the most dominant component with 58.65±0.95 %, followed by hexyl 2-methylbutyrate (10.61 %), hexyl butyrate (9.37±0.00 %), hexyl isobutyrate (6.01±0.71 %), octyl 2-methylbutyrate (3.18±0.17 %), hexyl hexanoate (2.23±0.25 %), octyl isobutyrate (1.63±0.08 %), and hexyl isovalerate (1.00±0.34 %) (Figure 2). All these compounds belong to aliphatic esters – the most dominant group in the Giant Hogweed seeds EO with an overall representation > 95 %, followed by monoterpenes (1.86 %), and sesquiterpenes (0.81 %). Aliphatic hydrocarbons, alcohols, aldehydes, and ketones were detected too, with an overall representation of 1.50 %.

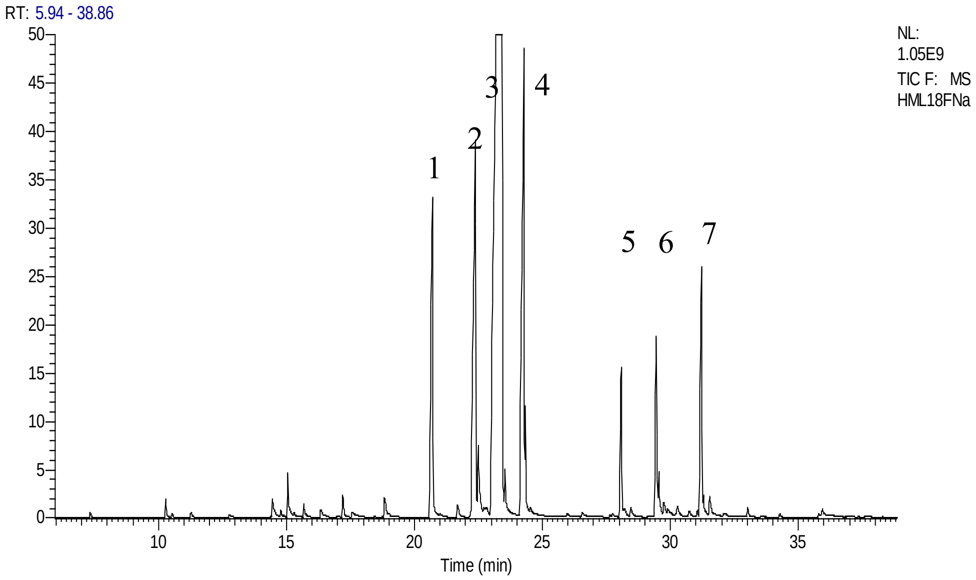


Figure 2. GC-MS chromatogram of essential oil of Heracleum mantegazzianum seeds. Peak identification: 1) hexyl isobutyrate, 2) hexyl butyrate, 3) octyl acetate, 4) hexyl 2-methylbutyrate, 5) octyl isobutyrate, 6) hexyl hexanoate, 7) octyl 2-methylbutyrate

Aliphatic esters are fragrant products found mainly in fruits and used as fragrance ingredients. Octyl acetate (octyl ethanoate) is found in oranges, grapefruits, and other citrus products as well as in many autumn fruits, including apples and pears. It has a typical orange aroma. Hexyl 2-methylbutyrate (hexyl 2-methylbuanoate) is found in apricot and has strong fruity notes. Hexyl hexanoate with green type odor is found in passion fruit, apples, strawberries, and wine.

Table 1. Chemical composition (%) ± SD of essential oil from seeds of *Heracleum mantegazzianum* Sommier et Levier

Chemical class	**Chemical Component**	**RI**	**RI l**	**Average content [%] ± SD**
Aliphatic esters	Octyl acetate	1204	1191	58.65 ± 0.95
	Hexyl 2-methylbutyrate	1227	1224	10.61 ± 0.57
	Hexyl butyrate	1177	1176	9.37 ± 0.00

	Hexyl isobutyrate	1133	1132	6.01 ± 0.71
	Octyl 2-methylbutyrate	1418	1421	3.18 ± 0.17
	Hexyl hexanoate	1367	1371	2.23 ± 0.25
	Octyl isobutyrate	1327	1329	1.63 ± 0.08
	Hexyl isovalerate	1228	1227	1.00 ± 0.34
	Octyl butyrate	1369	1371	0.65 ± 0.13
	Hexyl acetate	994	995	0.43 ± 0.11
	(E)-Oct-3-en-1-yl acetate	1207	1200	0.30 ± 0.03
	2-Methylbutyl octanoate	1428	1427	0.25 ± 0.02
	Hexyl propionate	1085	1085	0.24 ± 0.12
	Butyl 2-methylbutyrate	1026	1026	0.21 ± 0.04
	2-Methylbutyl 2-methylbutyrate	1087	1090	0.21 ± 0.00
	Octyl hexanoate	1567	1567	0.15 ± 0.04
	Decyl acetate	1390	1390	0.15 ± 0.02
	Octyl propionate	1285	1280	0.08 ± 0.00
	Isopropyl isobutyrate	781	783	t
	Butyl isobutyrate	937	939	t
	2-Methylbutyl isovalerate	1090	1094	t
	2-Methylbutyl hexanoate	1233	1235	t
	Oct-1-en-3-yl 3-methylbutyrate	1315	1315	t
Alkanes	Nonane	899	900	0.07 ± 0.02
	Decane	990	993	t
Aliphatic aLCohols	Octanol	1056	1063	0.19 ± 0.02
	Decan-2-ol	1188	1188	0.19 ± 0.00
	Heptan-2-ol	878	880	0.05 ± 0.01
Aliphatic aldehydes	Decanal	1180	1180	0.49 ± 0.30
	Octanal	979	982	0.36 ± 0.04
Aliphatic ketones	Heptan-2-one	871	871	0.15 ± 0.06
	Octan-2one	963	964	t
Monoterpenes	γ-Terpinene	1046	1055	0.51 ± 0.15
	p-Cymene	1009	1015	0.34 ± 0.11
	Isobornyl isobutyrate	1420	1424	0.20 ± 0.05
	Terpinen-4-ol	1159	1164	0.14 ± 0.05
	Lavandulyl acetate	1269	1270	0.15 ± 0.00
	α-Thujene	926	932	0.15 ± 0.00
	Isobornyl isovalerate	1513	1516	0.09 ± 0.05
	(E)-β-Ocimene	1042	1042	0.06 ± 0.04
	(Z)-β-Ocimene	1029	1029	0.07 ± 0.02
	α-Terpinyl acetate	1331	1335	0.07 ± 0.02
	Myrcene	987	987	0.08 ± 0.01
	α-Phellandrene	1000	1002	t
Sesquiterpenes	Germacrene D	1473	1479	0.22 ± 0.11
	δ-Elemene	1338	1340	0.12 ± 0.00
	(E)-β-Caryophyllene	1413	1418	0.11 ± 0.04
	β-Bourbonene	1379	1378	0.09 ± 0.01
	7-epi-α-Cedrene	1404	1404	0.09 ± 0.01

	Longipinanol	1563	1563	0.08 ± 0.04
	Isogermacrene D	1445	1445	t
	α-Humulene	1447	1455	t
Aliphatic esters				**95.66**
Other aliphatic compounds				**1.50**
Monoterpenes				**1.86**
Sesquiterpenes				**0.71**
Total identified				**99.73**

RIexp – experimental retention index, RIlit – literature retention index, t – trace (percentage value less than 0.05 %).

Our results are following the previous research (20), when EO from *H. mantegazzianum* aboveground parts was dominated by octyl acetate with a representation of 62.6 % as well, followed by hexyl 2-metylbutyrate (10.7 %), hexyl isobutyrate (7.5 %) and hexyl butyrate (6.5 %). Octyl acetate dominated EO from *H. mantegazzianum* (31) with representation 19.92 %, followed by n-hexyl-2-methylbutanoate (10.84 %), n-octanol (10.13 %), n-octyl butanoate (8.88 %), n-octyl-2-methylbutanoate (8.01 %), n-hexyl acetate (7.11 %), n-octyl isobutanoate (5.5 %) and n-hexyl isobutanoate (5.43 %). Similarly, octyl acetate was identified as one of the main EO compound in the other *Heracleum* genus representatives as well: in the seeds EO of *H. persicum* (Desf. ex Fischer) when its representation ranged between 11.2–20.3 % (23); in the EO from fruits of *H. sphondylium* (L. subsp. ternatum (Velen.) Brummitt.) (19); in the EO from aerial parts of *H. rechingeri* (Manden) with octyl acetate representation 29.49 % (Habibi 2010). A study of Tkachenko (35) analyzed the composition of *H. nanum* (Satzyperova), *H. leskovii* (Grossh.), *H. grandiflorum* (Stev. ex Bieb.), *H. moellendorfii* (Hance), *H. duLCe* (Fisch) EOs when octyl acetate had representations 41 %, 65 %, 39 %, 51 % and 40 % respectively.

The 3-hydroxybenzoic acid i.e. hydroxybenzoic acid derivatives, syringic acid - derivative of gallic acid with a role as a plant metabolite and chlorogenic acid - ester of caffeic acid and (−)-quinic acid functioning as an intermediate in lignin biosynthesis were the dominant polyphenols determined in the aqueous leachate from Giant Hogweed seeds (Table 2, Figure 3). The following other polyphenols were found to have a concentration less than 50 µg/g: caffeic acid (45.56±5.36 µg/g) - a secondary metabolite in the lignin biosynthesis, which occurs in almost all plants, ferulic acid (32.70±5.51 µg/g) - a natural compound in plant cells with antioxidant properties, p-coumaric acid (30.85±5.75 µg/g) - mainly a plant metabolite which exhibits antioxidant and anti-inflammatory properties, myricetin (30.69±5.07 µg/g) - polyphenolic compounds, with antioxidant properties and p-hydroxybenzoic acid (25.91±2.70 µg/g) - benzoic acid carrying a hydroxy substituent at C-4 of the benzene ring. Kaempferol, gallic acid, quercetin, and cinnamic acid were identified too, with a content lower than 20 µg/g.

Table 2. List and average content of polyphenols detected in the aqueous leachate from *H. mantegazzianum* seeds with the number of peaks at relevant chromatogram (Figure 3).

Peak number	**Chemical Component**	**Average content [µg/g] ± SD**
14	Cinnamic acid	12.24 ± 0.67
13	Quercetin	17.92 ± 1.92

1	Gallic acid	19.30 ± 2.80
16	Kaempferol	19.78 ± 0.98
4	p-Hydroxybenzoic acid (PHBA)	25.91 ± 2.70
11	Myricetin	30.69 ± 5.07
9	p-Coumaric acid	30.85 ± 5.75
10	Ferulic acid	32.70 ± 5.51
5	Caffeic acid	45.56 ± 5.36
3	Chlorogenic acid	125.92 ± 8.08
6	Syringic acid	140.15 ± 9.11
7	3-Hydroxybenzoic acid	158.14 ± 10.3
	Apigenin	N.D.
	Luteolin	N.D.
	Protocatechuic acid	N.D.
	Rutin	N.D.

N.D.: Not detected

Several of the polyphenols contained in the aqueous leachate were also identified in the other of *Heracleum* genus representatives, as f.e. caffeic, chlorogenic, and p-coumaric acids and quercetin in the seeds of *H. sphondylium* L. (7) or *H. persicum* (17). Overall polyphenols content of the *H. mantegazzianum* is similar to their content in *H. sosnowskyi* (Manden) (3).

Larvicidal Activity of Essential Oil, Octyl Acetate, and Aqueous Leachate

The results for *Ae. japonicus* larvae mortality after exposure to the essential oil from *H. mantegazianum* seeds, its main compound octyl acetate, and aqueous leachate after 24 h of exposure are shown in Table 3. The lethal concentration of the essential oil from *H. mantegazianum* seeds, its main compound octyl acetate, and the aqueous leachate from Giant Hogweed seeds based on Probit analysis are given in Table 4.

Table 3. Larvicidal activity of the essential oil from *H. mantegazzianum* seeds and the octyl acetate standard against *Ae. japonicus* larvae.

Concentration	**Accumulation of death**		**Mortality (%)**	
(mg/mL)	**EO**	**OA**	**EO**	**OA**
0.035	7.3±5.4	7.0±2.0	24.3	22.28
0.060	11.3±3.52	19.0±4.0	45.2	62.74
0.085	21.0±4.7	27.3±1.5	81.9	90.97
0.110	19.7±5.5	27.3±1.5	76.6	90.97
0.135	26.7±3.3	30.0±0.0	89.2	100.0

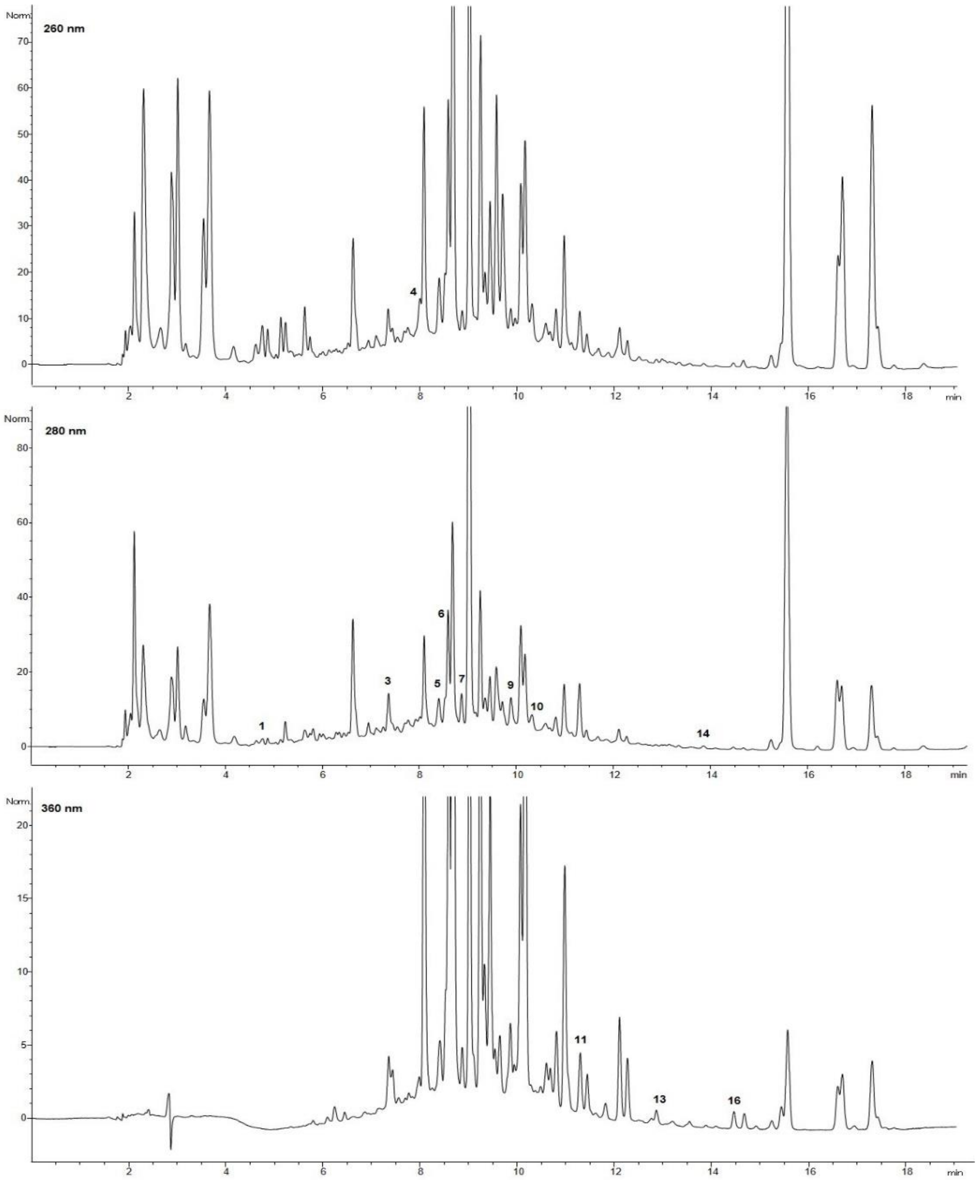


Figure 3. HPLC-DAD chromatograms of polyphenols detected in the aqueous leachate from *H. mantegazzianum* seeds. Peak identification: 1) Gallic acid, 3) Chlorogenic acid, 4) P-hydroxybenzoic acid, 5) Caffeic acid, 6) Syringic acid, 7) 3-Hydroxybenzoic acid, 9) p-Coumaric acid, 10) Ferulic acid, 11) Myricetin, 13) Quercetin, 14) Cinnamic acid, 16) Kaempferol.

EO and OA caused significantly ($p < 0.05$) higher mortality of *Ae. japonicus* in comparison to control by all tested concentrations. Mortality of the Asian bush mosquito was, in contrast to the control, significantly ($p < 0.05$) higher when using aqueous leachates with concentrations higher than 10 mg/mL only. Octyl acetate itself was the most effective against *Ae. japonicus* larvae, followed by the essential oil, while aqueous leachate had the least mortality. Positive significant dependence was observed between Asian bush mosquito larvae mortality and EO ($R=0.95$, $p < 0.001$), OA ($R=0.95$, $p < 0.01$), and aqueous leachate ($R=0.93$, $p < 0.001$) concentrations.

The essential oil showed a larvicidal effect against *Ae. japonicus,* but, equally against *Chi. aprilinus* and *Chaoborus* spp. However, Asian bush mosquito mortality was significantly higher in comparison to glass worms and blood worms by all EO and OA concentrations. Concerning aqueous leachate, *Ae. japonicus* larval mortality was considerably higher in comparison to those of glass worms and blood worms by concentrations lower than 10 mg/mL; by the concentrations over the 14 mg/mL, mortality of all organisms was balanced.

Table 4. LC_{50}, LC_{90} and Chi test ($\chi 2$) values of *H. mantegazzianum* seeds essential oil, essential oil main compound Octyl acetate and *H. mantegazzianum* seeds aqueous leachate larvicidal activity against mosquito *Ae.japonicus* and non-target organisms *Chaoborus* spp. and *Chi. aprilinus.* NS means that there were no statistically significant differences. SIG means statistically significant differences.

Essential oil	**LC_{50} LCL–UCL (95 % confidence limit) mg/mL**	**LC_{90} LCL–UCL (95 % confidence limit) mg/mL**	**Chi test ($\chi 2$)**	**P value**	**df**
Ae. japonicus	0.067 (0.05-0.088)	0.179 (0.135-0.239)	0.637	NS	3
Chaoborus spp.	0.143 (0.105-0.193)	0.406 (0.300-0.550)	0.008	SIG	3
Chi. aprilinus	0.160 (0.137-0.186)	0.207 (0.178-0.241)	-	-	0
Octyl acetate	**LC_{50} LCL–UCL (95 % confidence limit) mg/mL**	**LC_{90} LCL–UCL (95 % confidence limit) mg/mL**	**Chi test ($\chi 2$)**	**P value**	**df**
Ae. japonicus	0.052 (0.041-0.065)	0.103 (0.082-0.130)	0.943	NS	2
Chaoborus spp.	0.117 (0.090-0.152)	0.293 (0.226-0.379)	0.004	SIG	3
Chi. aprilinus	0.152 (0.121-0.191)	0.294 (0.233-0.369)	0.32	NS	2
Aqueous leachate	**LC_{50} LCL–UCL (95 % confidence limit) mg/mL**	**LC_{90} LCL–UCL (95 % confidence limit) mg/mL**	**Chi test ($\chi 2$)**	**P value**	**df**
Ae. japonicus	9.235 (7.144-11.94)	32.88 (25.44-42.50)	0.284	NS	8
Chaoborus spp.	22.29 (13.81-35.97)	250.5 (155.3-404.2)	0.030	SIG	7
Chi. aprilinus	27.19 (16.25-45.50)	228.8 (136.7-382.9)	0.001	SIG	5

Until the MS submission, we have found the only study (27) that evaluated Ae's *japonicus* larvae mortality after the exposition to clove essential oil, since the used concentrations range was as follows: 0.010, 0.020, 0.040, 0.080, 0.160, 0.320 mg/mL, and the following results were obtained: LC_{50} was 0.017 mg/mL, and LC_{90} was 0.047 mg/mL. 100 % larvae mortality was observed at 0.080 mg/mL. In comparison, EO from

H. mantegazzianum seeds shoved in our study about three times lover larvicidal activity against *Ae. japonicus*.

Our results are also comparable to the larvicidal activity of the essential oils from the other *Heracleum* genus representatives, which were tested for their mosquito larvae toxicity too: EO from *H. persicum* seeds against *Anopheles stephensi* (Liston, 1901) (29) with LC_{50} 0.020 mg/mL and LC_{90} 0.045 mg/mL; EO from *H. sprengelianum* (Wight & Arn.) leaf against *An. subpictus* (Grassi, 1899), *Ae. albopictus* (Skuse, 1894) and *Culex tritaeniorhynchus* (Giles, 1901) (15) with LC50 of 0.033, 0.038, and 0.041 mg/mL respectively; EO from *H. pastinacifolium* subsp. *transcaucasicum* ((Manden.) P.H. Davis) and *H. pastinacifolium* subsp. *incanum* ((Boiss. and A. Huet) P.H. Davis) fruits against *Ae. aegypti* (Linnaeus, 1762) (34) showing LC_{50} 0.069 mg/mL and 0.072 mg/mL and LC_{90} 0.152 and 0.179 mg/mL respectively.

Larvicidal impact of Temephos – synthetic organophosphate used to treat water infested with disease-carrying insects, including mosquitoes, against *An. stephensi* and *Cx. quinquefasciatus* (Say, 1823) (37) was higher then those of the EO from *H. mantegazzianum* seeds or octyl acetate. But, the lower when using against *Ae. aegypti* (33).

As mentioned in the introduction, to our knowledge, aqueous leachate from the *H. mantegazzianum* seeds was tested for its *Ae. japonicus* larvicidal activity for the first time. However, several studies had confirmed insecticidal activity of the same polyphenols, which were detected in the Giant Hogweed seeds aqueous leachate in our study: cinnamic acid and its derivates showed larvicidal activity against *Ae. aegypti* (2). A study of *Parthenium hysterophorus* L. extracts confirmed its larvicidal solid activity against *Ae. aegypti* too, since chlorogenic acid and caffeic acid were detected in its extracts (1). The same concerned ferulic acid in connection to *Cx. pipiens* Linnaeus, 1758 (36), quercetin, myricetin and kaempferol in connection to *Ae. aegypti* or *Cx. quinquefasciatus* (26). A study by Younus *et al*. (39) confirmed the strong insecticidal activity of the *Euphorbia nivulia* L. extracts, which similarly contained gallic, caffeic, syringic, coumaric, ferulic, and cinnamic acids as well as quercetrin. The same concerns also hydroxybenzoic acid (11). Study of Subba (32) evaluated different extracts of the fruits of *H. nepalense* (D. Don) Manden), against larvae of *Ae. albopictus,* since almost all the solvent extracts showed some amount of mortality. Diethyl ether extract showed 100 % mortality at 0.2 mg/mL followed by n-hexane, acetone, and methanol extracts.

CONCLUSIONS

Our study showed that biologically active substances from invasive Giant Hogweed *Heracleum mantegazzianum* has larvicidal potential and can be better explored to control the Asian bush mosquito (*Aedes japonicus*) as well as other of flying, blood sucking potential vectors of serious human disease. Simultaneously, essential oil, octyl acetate as well as aqueous leachate caused just moderate mortality of non-target organisms what gives a good basement for the practical application within an environment without harmful effect on wild living organisms.

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DECLARATION

We declare that all authors of this Ms. have made substantial contributions. We did not exclude any author who substantially contributed to this Ms. We have followed our ethical norms established by our respective institutions.

CONFLICT OF INTEREST

The authors announce that they have no conflict of interest.

ETHICAL APPROVAL

The authors declare that the study was carried out following scientific ethics and conduct. However, this study did not involve any use of animals, hence no ethical approval has been obtained from the concerned committee.

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