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Chemical composition and biological activities of *Valeriana dioscoridis* SM. roots

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ABSTRACT

Zika, dengue, and Yellow fever are transmitted primarily by *Aedes aegypti* mosquitoes. Investigations to find more natural but highly effective pesticidal agents against *Ae. aegypti* have increased in the last few years. Plant-based compounds have been the source of several current pesticides and have provided models for creation of synthetic derivatives like the pyrethroids. Plants continue to be a rich source for potential new active ingredients. The aim of this study is to increase knowledge on *V. dioscoridis* and to present the results of the cytotoxic and insecticidal activities of various extracts of its roots and volatile compound compositions of roots. The hexane and chloroform extracts, HM1 and CM1 respectively, exhibited cytotoxicity with IC_{50} values of 128.4 and 86.93 μg/mL against the HepG2 cell line. Because CM1 had higher cytotoxicity across the studied concentration range, it was also tested against the HUVEC cell line. Extracts HM1 and CM1 were also tested for their insecticidal activities. CM1 was found to be the more active (mortality (%) = 96.7% ± 5.8 at 5 μg/mosquito), consistent with its cytotoxic activity. Since these active extracts were nonpolar, the chemical composition of the volatile compounds of the roots were analyzed by GC-MS analysis. About 87.7% of the essential oil were characterized. The major compound was ß-eudesmol (9.2%), following by camphene (8.9%), bornyl acetate (8.2%) and maaliol (7.6%). This is the first study on the HepG2 and HUVEC cytotoxicity and insecticidal activity of *V. dioscoridis*. Further studies on the potential of this plant to define the spectrum of activity and examine potential therapeutic agents is warranted.

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1. Introduction

Zika virus, is a disease with symptoms including skin rash, mild fever, muscle and joint pain, conjunctivitis, malaise or a headache, and severe morbidities like microcephaly and Guillain-Barré syndrome. It is transmitted primarily by *Aedes* mosquitoes. In recent years, the researchers increased their investigations also for the aim of the finding and effective agent against *Ae. aegypti*. (WHO, 2021).

Due to the negative and undesirable effects of synthetic agents, the development of drug resistance, and negative public opinion toward synthetic pesticides, there is a desire to identify more natural therapeutic agents. Investigations on plants and herbal preparations, which have been used in traditional medicine for thousands of years with a variety of therapeutic functions, continue to demonstrate that they can have beneficial effects. Studies of the efficacy of natural plant extracts as pesticides are increasing to find new, highly

This work is dedicated to Prof. Dr. Afife Mat.

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effective agents against *Ae. aegypti* (Tabanca et al., 2016; Castillo et al., 2017; Abu Bakar et al., 2018; Demiray et al., 2019).

The *Valeriana* species (Caprifoliaceae) are known as 'kediotu' in Turkey. *Valeriana officinalis* is traditionally preferred for the treatment of nervous insomnia, palpitations, neurasthenia, hysteria and neural diseases. Infusions are consumed for the treatment of wounds in traditional Turkish medicine (Aslan, 2012; Baytop, 1999). Previous studies have shown *Valeriana* extracts can contain over 150 chemical constituents such as iridoids, flavonoids, alkaloids, lignans, and terpenoids. The antispasmodic, sedative, antimycobacterial, antiviral, cytotoxic and anxiolytic activities of many iridoids of *Valeriana* species were determined. The valepotriate iridoids are widespread in the *Valeriana* species, and are cytotoxic due to inhibition of DNA synthesis (Wang et al. 2010; Sen and Mat, 2015). *In vitro* studies on the cytotoxic activities of the extracts and isolated compounds, such as iridoids, sesquiterpenoids, from *Valeriana* species against various cell lines exist in the literature (Aydın et al., 2016; Liu et al., 2017). Some investigations of the insecticidal activities of *Valeriana* species against mosquitoes and moths have been conducted (Dua et al., 2008; Lee et al., 2018; Sen-Utsukarci et al., 2019).

In Turkey, the usages of *Valeriana dioscoridis* SM., known as "kediotu, pisiotu, pisikotu, düllüdamak", were recorded in ethnobotanical studies. The aerial parts are used for wound healing, as a sedative, and antispasmodic. The infusion of its flowers is used for lowering tension (Bulut, 2006; Poyraz-Kayabasi et al., 2018; Demirci and Ozhatay, 2012).

Studies on *V. dioscoridis* SM. are limited as few have been conducted. In the first investigation of this species, essential oils from its aerial parts and roots were studied for their chemical composition using GC/MS and antifungal activities. The major compound of the essential oils from aerial parts and roots was patchouli alcohol (Tzakou et al., 2004). Additionally, its antioxidant activity was evaluated along with other Turkish medicinal plants (Karadeniz et al., 2015). The chemical composition and antiproliferative effects of the methanol extract of *Valeriana dioscoridis* roots was determined by Kirmizibekmez et al. Among the compounds isolated, all 7 iridoids exhibited modest activity against human cancer cell lines (HeLa, A2780 and T47D), in comparison to cisplatin (Kırmızıbekmez et al., 2018). In two recent studies, the ethanol (80%) extract of its subterranean parts were studied for its antioxidant and antimicrobial effects, and the methanol, ethyl acetate extracts and the infusion from its aerial parts for their antioxidant and tyrosinase inhibitor activities (Ucar et al., 2020; Sarikurkcu et al., 2020).

The aim of this study is to further develop knowledge on *V. dioscoridis* and to present the results of the cytotoxic and insecticidal activities of various extracts and volatile chemical compositions of its roots.

2. Materials and methods

2.1. Plant material

The roots of *Valeriana dioscoridis* were collected from Kahramanmaras – Goksun (Turkey). The voucher specimen has been prepared and authenticated by S. Demirci and deposited in the Herbarium of the Faculty of Pharmacy, Cukurova University (CUEF 754).

2.2. Preparation of extracts

The dried and powdered roots were successively macerated with hexane (HM1), chloroform (CM1), and ethanol (EM1) and water (WM1), with stirring for 24 h. Furthermore, two portions of the aerial parts were individually macerated with ethanol (EM2) and water (WM2), with stirring for a day. An infusion was prepared from another portion with boiled water (WI) in order to mimic its traditional use. The organic extracts were evaporated to dryness and the aqueous extracts were lyophilized. The extracts were stored at ± 4 °C until use.

2.3. Biological assays

2.3.1. Cytotoxicity assay

The cytotoxicity tests on human liver carcinoma [HepG2; HB-8065, controlled by DSMZ-German Collection of Microorganisms and Cell Cultures GmbH, Germany (DSMZ ACC180)] and primary human umbilical vein endothelial [HUVEC; isolated from umbilical cords (Umbilical cords were obtained with the consent of patients and permission by the local ethics committee)] cells, were performed using the MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] colorimetric method described in our previous investigation (Sen-Utsukarci et al., 2019).

The percentage cell viability was calculated with respect to solvent control as follows:

$$\%\text{Cellviability} = \text{Abs}_{\text{Compounds}}/\text{Abs}_{\text{SolventControl}}\text{x}100$$

2.3.2. Selectivity index (SI)

Selectivity index expresses selective toxicity against cancer cells. The compounds with SI less than 2 are considered generally toxic even to healthy cells. The SI values higher than 2 are considered to have selective toxicity against cancer cells (Badisa et al., 2006). The SI value was calculated using the formula:

$$SI = IC_{50}normalcell/IC_{50}cancercell$$

2.3.3. Insecticidal activity assay

The mosquito colony of *Aedes aegypti* L. was obtained from the USDA-ARS-CMAVE insectary (Gainesville, FL, USA). Rearing procedures have been described elsewhere (Tabanca et al., 2016).

For the larvicidal and adulticidal activity, the tests were performed essentially as described in our previous study (Sen-Utsukarci et al., 2019). The extracts were dissolved in DMSO with gentle heating if needed. In the larval assays, mortality was determined at four different concentrations (1.0, 0.5, 0.25, and 0.1 μg μL^{-1}). The DMSO solubilized extracts were evaluated against adult *Ae. aegypti* using cohorts of 3-6-day post-emergence females exposed to 5ug uL^{-1} in acetone. After treatment, mosquitoes were kept in plastic cups at 26–27 °C and 80 % humidity after treatment and supplied with 10 % sucrose in water for 24 h prior to recording mortality. For each assay, a positive control of permethrin stock (permethrin mixture of 46.1 % *cis*- and 53.2 *trans*-isomers (Chemservice, West Chester, PA)) and a negative control of DMSO or acetone was included. All insecticidal bioassays were replicated three times.

2.3.4. Statistical analysis

The IC50 values were calculated as mean ± standard error of the mean (SEM) by using curve fitting software (GraphPad Prism version 8). The statistical significance were analyzed by one-way ANOVA followed by Tukey's post hoc test for multiple comparisons. Probability levels of less than 0.05 ($p < 0.05$) were considered significant.

2.4. GC and GC-MS analysis of the chemical composition

Dried and crushed roots were microdistilled as described in our previous study (Sen-Utsukarci et al., 2019). After completion of distillation, the organic layer in the collection vial was injected into the GC/GC-MS instrument.

The essential oils were analysed with GC-FID and GC/MS techniques simultaneously. The GC-FID analysis was carried out with a capillary Agilent 6890N GC system and the GC-MS analysis was

performed using an Agilent 5975 GC-MSD system. FID temperature was set at 300 °C in order to obtain the same elution order with the GC-MS. Injection was simultaneous using the same HP-INNOWax FSC column (60 m × 0.25 mm, 0.25-μm film thickness; Agilent, Walt & Jennings) and appropriate operational conditions. The components of essential oils were identified by comparison of their mass spectra with those in the Baser Library of Essential Oil Constituents, Adams Library, MassFinder Library, Wiley GC/MS Library and confirmed by comparison of their retention indices (Adams, 2007; Hochmuth, 2008; McLafferty and Stauffer, 1989). Relative quantities (in percent) of the separated compounds were calculated from the FID chromatograms.

3. Results and discussion

Previous investigations on compounds from *Valeriana* species have examined sedative and anxiolytic effects as well as the effects on the GABA neurotransmitter receptor system (Tabanca et al., 2016). There have also been studies of cytotoxicity against cancer cell lines. Tinctures, prepared from the subterranean parts of *V. officinalis* L., *V. edulis* Nutt. ex Torr & Gray ssp. *procera* (H.B.K.) F. G. Meyer (*V. mexicana* DC.) and *V. wallichii* DC. (*V. jatamansi*), and the isolated constituents were studied for their cytotoxic effect against human small-cell lung cancer cell line (GLC4) and human colorectal cancer cell line (COLO320) by MTT assay. The diene type valepotriates -acetovaltrate, isovaltrate and valtrate- were 2- to 3-fold more toxic than the valepotriates of the monoene type -didrovaltrate and isovaleroxyhydroxydidrovaltrate-. The decomposition products of valepotriates (baldrinal and homobaldrinal) exhibited 10- to 30-fold less cytotoxicity and isovaltral higher cytotoxicity. Additionally, valerenic acid and derivatives like, acetoxyvalerenic acid, hydroxyvalerenic acid and methyl valerenate elicited low cytotoxicity (IC_{50} =100–200 μM) (Bos et al., 1998). In our previous study on the activity of the extracts from *V. alliariifolia* roots, the hexane extract prepared by gradient maceration and the ethanol extract prepared by maceration were found to be the most active extracts against HepG2 and HUVEC cell lines (IC_{50}: 52.42, 51.63 μg/mL, respectively). The chloroform extracts were less active (Sen-Utsukarci et al., 2019). In this present study, the extracts EM1, WM1, EM2, WM2 and WI were not cytotoxic at the studied concentrations (Table 1). The test could not be repeated at low concentration (<10 μg/mL) due to the limited quantity of extracts, but it would be beneficial to continue these studies with lower concentrations. At the studied concentrations, the extracts HM1 and CM1 exhibited cytotoxic activity on the HepG2 cell line (Table 1). CM1 (IC_{50}: 86.93 μg/mL) was more toxic than HM1 (IC_{50}: 128.4 μg/mL). On the basis of these initial evaluations, CM1 extract had higher activity than HM1. Therefore, the CM1 was further investigated using HUVEC cells as a model for healthy cells. The IC_{50} values of CM1 against HUVEC cell lines was 160.3 μg/mL. CM1 is effective on the cancer cells (HepG2 cells), but not effective against healthy cells (HUVEC cells), at the concentration of 86.93 μg/mL. The polar extracts of *V. dioscoridis* roots are more active than its nonpolar extracts, while the results of previous studies show that polar extracts are active, as well as very good results in nonpolar extracts. Additionally, the nonpolar extracts (HM1 and CM1) of *V. dioscoridis* demonstrated less cytotoxic activity against both of HepG2 and HUVEC cell lines than the nonpolar extracts obtained from *V. alliarifolia* roots. CM1 was toxic against both of cancer and normal healthy cell lines (SI < 2; Table 1). Since the selectivity value is close to two, we estimate that it will be possible to discover selective effective agents in future studies of the extract. More selective cytotoxic extracts and compounds with higher compliance in the treatment may be detected by investigation on other extracts at a concentration lower than 10 μg/mL.

Table 1
The IC_{50} values (μg/mL) of the extracts in MTT

	IC_{50} (HepG2)	IC_{50} (HUVEC)	SI
HM1	128.4	*	*
CM1	86.93	160.3	1.84
EM1	<10	*	*
WM1	<10	*	*
EM2	<10	*	*
WM2	<10	*	*
WI	<10	*	*

* have not been measured; SI=Selectivity index

Treatment with all extract concentrations had significant cytotoxic effects ($p < 0.001$, Fig. 1). More cytotoxicity was seen in the 10 and 25 mg/ml concentration of HM1 on the HepG2 cell line than in the other concentrations ($p < 0.001$, Fig. 2). In comparison to the concentration of CM1 on HepG2 cell line, CM1 induced a concentration-dependent effect and the 100 and 200 mg/ml concentration were the most cytotoxic when compared to the others, as would be expected ($p < 0.001$, Fig. 2). Like the HepG2 result, CM1 induced a concentration-dependent effect on HUVEC cell line and the 100 and 200 mg/ml concentration were most cytotoxic compared to others ($p < 0.001$, Fig. 2).

We also examined insecticidal activity because previous studies with *Valeriana* species are limited but promising. In one study, the lethal concentration (LC_{50}) of *V. jatamansi* essential oil against larvae of *Culex quinquefasciatus, Anopheles stephensi, Aedes albopictus, Ae. aegypti* and *Anopheles culicifacies* were 80.6, 68.1, 53.8, 51.2 and 42.8 mg/L, respectively. The LC_{50} against adult *C. quinquefasciatus, A. culicifacies, A. stephensi, Ae. aegypti* and *A. albopictus* were found as 0.17, 0.16, 0.14, 0.09 and 0.08 mg/cm^2, respectively (Dua et al., 2008). Similarly, *V. alliariifolia* also has insecticidal activity. Extracts were tested against adult and larvae *Ae. aegypti*. This demonstrated differential activity against different life stages. The extracts were active against adult *Ae. aegypti*, but no extracts were active against larvae. Mortalities of the hexane, chloroform and ethanol extracts was respectively 90 ± 10%, 73.3 ± 25.2% and 76.7 ± 15.3% at a dose of 5 μg extract/ mosquito (Sen-Utsukarci et al., 2019). The cytotoxic extracts were also tested for insecticidal activities (adult topical activity and larvicidal activity; Table 2). CM1 was more active extract than HM1, in parallel to its cytotoxic activity. Compared to the results of previous insecticidal activity assays, *V. dioscoridis* was more active than other species. CM1 exhibited activity like HM1, but with much better mortality.

All extracts had significant insecticidal activity when compared to untreated and negative control groups ($p < 0.001$, Fig. 3). In comparison to the activity of the positive control, all extracts had significantly higher insecticidal activity than the 0.24 ng dose of permethrin. There was no significant difference between the activities of these extracts and permethrin at high concentration of 2.54 ng. It suggests that CM1 at 5ug has about the same effect as permethrin at 2.54 ng.

Since the extracts were nonpolar, the chemical composition of the microdistilled root essential oil was analyzed by GC-MS (Table 3).

In GC-MS analysis (Table 3), 42 compounds constituting about 87.7 % of the essential oil of *V. dioscoridis* roots were characterized. The major compound was found ß-eudesmol (9.2%), following by camphene (8.9%), bornyl acetate (8.2%) and maaliol (7.6%). In a previous GC-MS study on the chemical composition of the hydrodistilled essential oils from the aerial parts and subterranean parts of *V. dioscoridis*, the major compounds of the subterranean parts were patchouli alcohol (13.4%), by following α-pinene (8.6%), selina-4(15),7(11)-diene (7.0%), β-pinene (7.0%), α-fenchene (5.2%) and 2,5-dimethoxy-p-cymene (4.8%) (Tzakou et al., 2004). Ucar et al. also analyzed the chemical composition of the ethanol (80%) extract from the subterranean parts. The compounds, 9-[3-(dimethylamino) propyl]-9-borabicyclo [3.3.1] nonane (17.55%), oleic acid amide (8.22%) and (1,3-butadienylidene)-cyclohexane (7.89%) were determined to be the

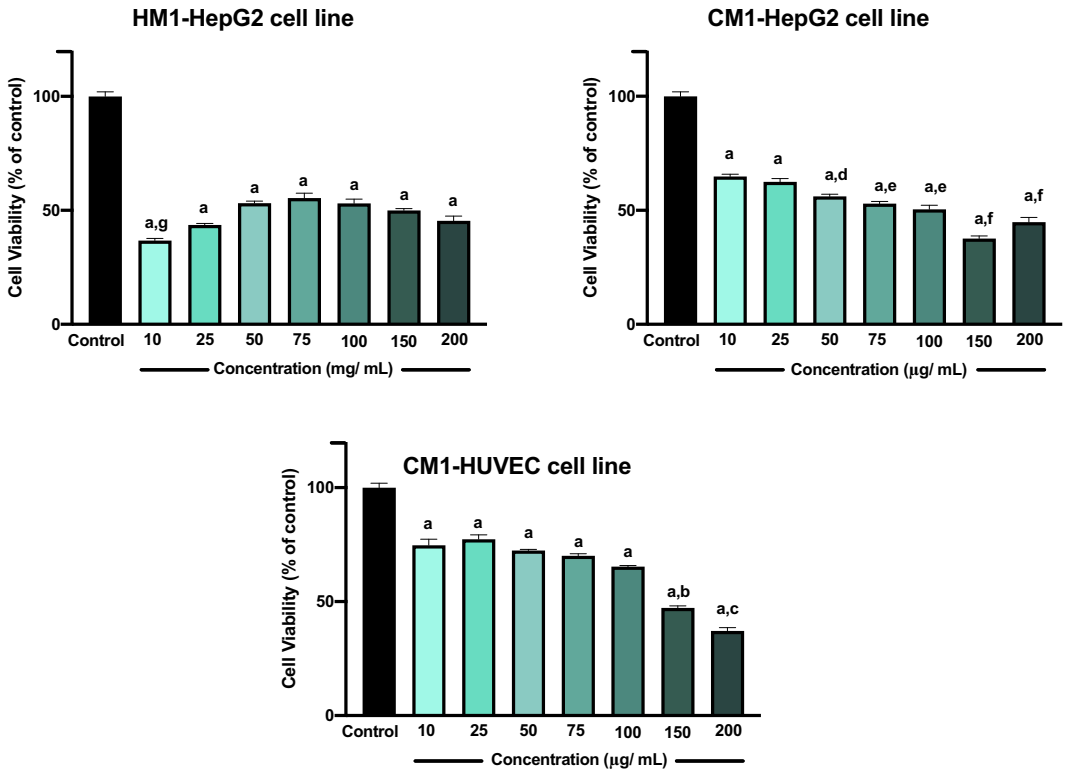

Fig. 1. The cytotoxic effects of HM1 and CM1 extracts of *Valeriana dioscoridis* on HepG2 and HUVEC cell lines

The cytoxic effects of each HM1(A) and CM1 (B) after serial dilution (10–200 mg/ml) were measured on HepG2 cell line. Also, cytoxic effect of CM1 concentrations on HUVEC cell line were evaluated (C). Data are given as mean ± SEM of 6–11 repetitions.

[a] $p < 0.0001$; vs control

[b] $p < 0.01$ vs 0.01, 0.025, 0.05, 0.1 mg/ mL concentration of CM1 at HUVEC cell line

[c] $p < 0.001$ vs 0.01, 0.025, 0.05, 0.1 mg/mL concentration of CM1 at HUVEC cell line

[d] $p < 0.05$ vs 0.01, 0.025 mg/mL concentration of CM1 at HepG2 cell line

[e] $p < 0.01$ vs 0.01, 0.025, 0.05 mg/ ml concentration of CM1 against HepG2 cell line

[f] $p < 0.001$ vs 0.01, 0.025, 0.05, 0.1 mg/mL concentration of CM1 against HepG2 cell line

[g] $p < 0.001$ vs 0.05, 0.1, 0.175, 0.15, 0.2 mg/mL concentration of HM1 at HepG2 cell line.

major components (Ucar et al., 2020). This variability in the composition of volatile compounds depending on the plant collection in different locations, extraction method, and different time periods is reflected in these varied results.

A number of investigations of the activity of these major constituent compounds or extracts containing high amounts of them have been reported. Extracts or essential oils containing high amounts of ß-eudesmol, bornyl acetate and maaliol elicited a good cytotoxic *in*

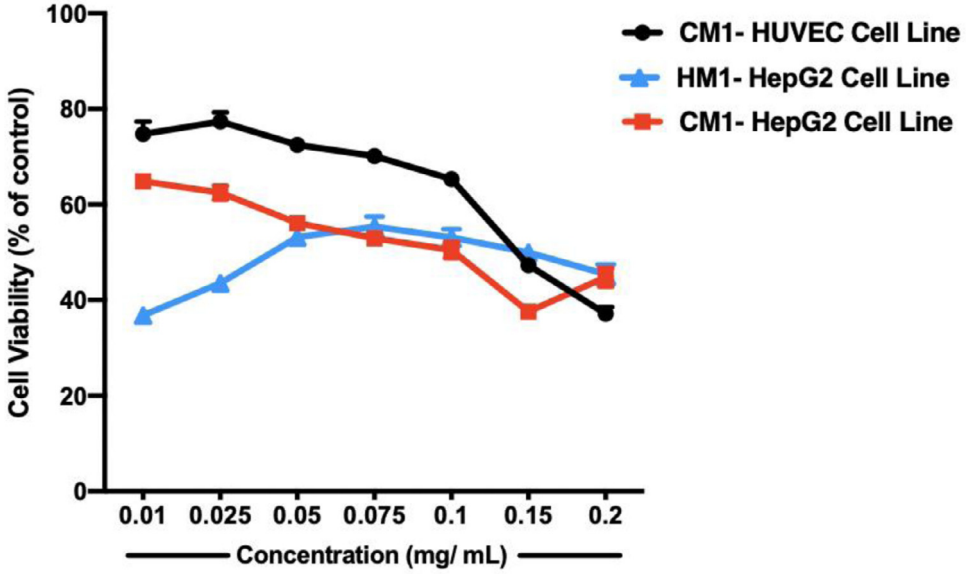

Fig. 2. Comparison between the cell viability of HepG2 and HUVEC cell lines after the treatment with HM1 and CM1 extracts

Table 2
The mortalities of the extracts HM1 and CM1 against adult female mosquito *Ae. aegypti* and 1st instar *Ae. aegypti* larvae

Code	Adult female mosquito *Aedes aegypti* 5 μg/mosquito	1st instar *Aedes aegypti* larvae			
		1 μg/μL	0.5 μg/μL	0.25 μg/μL	0.1 μg/μL
HM1	83.3 ± 11.5	0	0	0	0
CM1	96.7 ± 5.8	0	0	0	0

Positive control permethrin at 0.24 ng/mosquito resulted in 66.7 ± 3.3 % mortality and at 2.54 ng/mosquito resulted in 100 ± 0% mortality; negative control (solvent control - acetone) had 3.3 ± 3.3 % mortality.

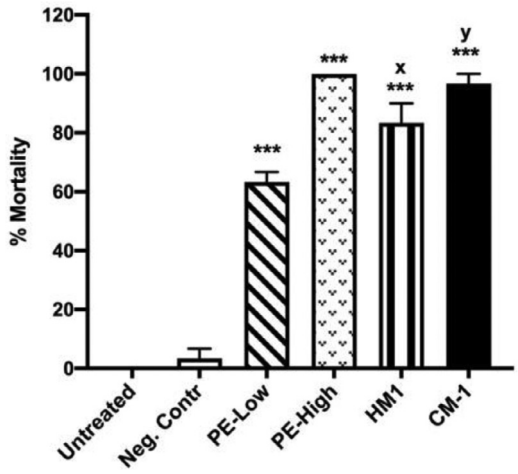

Fig. 3. The mortalities of the HM1 and CM1 extracts against adult female mosquito *Ae. aegypti*; PE-Low: permethrin at low concentration (0.24 ng/mosquito); PE-High: permethrin at high concentration (2.54 ng/mosquito);
*** $p < 0.001$ vs untreated or negative control group; x: $p < 0.05$ vs PE-Low; y: $p < 0.001$ vs PE-Low

vitro and *in vivo* (Britto et al., 2012; Karan et al., 2018; Ibrahim et al., 2019; Mothana et al., 2018; Amina et al., 2018; Azeem et al., 2019). Synergistic effects of the essential oil components from 5 cytotoxic plants against MCF-7 cells were investigated by Wright et al. The isolated compound camphene antagonized the cytotoxic activity of α-humulene and reduced the cytotoxicity of β-caryophyllene (Wright et al., 2007). Bornyl acetate had a cytotoxic activity on several cancer and healthy cell lines (IC_{50}: 72.0 μg/mL against HeLa; 60.5 μg/mL against HT29; 44.1 μg/mL against A549; 85.6 μg/mL against MCF-7; 50.6 μg/mL against FL) (Karan et al., 2018). Maaliol, isolated from its ethylacetate extract of *Plectranthus cylindraceus*, was found to be cytotoxic against human breast (MBDMB321), skin (HT1080), cervix (HeLa) cancer cells with greater cytotoxicity than the doxorubicin control (Amina et al., 2018). Also in a study on cytotoxicity of valerian tinctures and constituents, it was emphasized that some components in the *Valeriana* essential oils, such as maaliol, patchouli alcohol and cryptofauronol, possess cytotoxic activity comparable to valerenic acid analogs (Bos et al., 1998). Studies on ß-eudesmol, a sesquiterpenoid alcohol, have mainly focused on the cytotoxicity and mechanism of action of the compound (Li et al., 2013; Plengsuriyakarn et al., 2015; Mathem et al., 2017; Kotawong et al., 2018; Srijiwangsa et al., 2018). It exhibited a cytotoxic activity after 72 h of treatment on HL60 cell lines with the IC_{50} value 35.1 μM (Li et al., 2013). In a study comparing the cytotoxicity of the eudesmol isomers against human hepatocellular carcinoma (HepG2), human chronic myelocytic leukaemia (K562) and mouse melanoma (B16-F10), it was determined that α-eudesmol (IC_{50} values of 9.71, 10.60 and 5.38 μg/mL, respectively) had the highest cytotoxic potential, while ß-eudesmol was less effective (the IC_{50} values

Table 3
Composition of the essential oil isolated by microdistillation of the roots.

RRI	Compounds	%	IM Identification Metod
1032	α−Pinene	5.0	t_R, MS
1076	Camphene	8.9	t_R, MS
1118	β-Pinene	3.2	t_R, MS
1203	Limonene	1.0	t_R, MS
1255	γ-Terpinene	0.1	t_R, MS
1280	*p*-Cymene	1.1	t_R, MS
1290	Terpinolene	0.1	t_R, MS
1495	Bicycloelemene	0.1	MS
1586	Isothymol methyl ether	1.3	MS
1590	Bornyl acetate	8.2	t_R, MS
1600	β-Elemene	0.4	MS
1610	Calarene (= β-gurjunene)	1.3	t_R, MS
1612	β-Caryophyllene	2.6	t_R, MS
1614	Carvacrol methyl ether (= methyl carvacrol)	0.3	t_R, MS
1651	γ-Elemene	tr	MS
1661	Alloaromadendrene	tr	MS
1684	Isovaleric acid	3.7	MS
1687	α-Humulene	0.4	t_R, MS
1690	Selina-4,11-diene (= 4,11-eudesmadiene)	0.3	MS
1706	α-Terpineol	0.6	t_R, MS
1718	Borneol	1.3	t_R, MS
1719	γ-Guaiene	0.2	MS
1740	β-Selinene	0.7	MS
1744	α-Selinene	0.9	MS
1751	Bicyclogermacrene	0.3	MS
1772	δ-Cadinene	0.3	t_R, MS
1815	Valeric acid	0.1	MS
1878	2,5-Dimethoxy-p-cymene	1.1	MS
1882	1-Isobutyl 4-isopropyl-2,2-dimethyl succinate	1.2	MS
2008	Caryophyllene oxide	3.6	t_R, MS
2012	Maaliol	7.6	MS
2041	(*E*)-nerolidol*	3.1	t_R, MS
2096	Elemol	1.6	MS
2113	Guaiyl acetate	1.0	MS
2144	Spathulenol	0.8	t_R, MS
2157	Neointermedeol	0.4	MS
2191	γ-Eudesmol	6.8	MS
2246	α-Eudesmol	6.9	MS
2255	β-Eudesmol	9.2	MS
2273	Selin-11-en-4α-ol	2.1	MS
2316	Caryophylladienol I	0.5	MS
2352	Caryophyllenol I (= caryophylla-2 (12),6-dien-5α-ol)	0.4	MS

RRI, Relative retention indices calculated against *n*-alkanes; %, calculated from the FID chromatograms; tr, trace (<0.1 %); identification method: tR, identification based on the retention times (tR) of genuine compounds on the HP Innowax column; MS, identified on the basis of computer matching of the mass spectra with libraries and comparison with literature data-RRI.
*impure

of 24.57, 22.50 and 16.51 μg/mL, respectively) than other isomers. This study also investigated their effectiveness on peripheral blood mononuclear cells. The IC_{50} value of ß-eudesmol was higher than the other isomers suggesting that it can be used more safely (Bomfim et al., 2013). In the light of all these data, the volatile compounds of *V. dioscoridis* roots have a role in the cytotoxicity of the plant. The high activity of the studied extracts is an indication that the plant contains more cytotoxic compounds than just the major constituents of its essential oil.

Investigation of the insecticidal activity of ß-eudesmol, camphene, bornyl acetate and extracts or essential oils containing them against *Ae. aegypti* and other insects have been conducted previously (Kordali et al., 2007; Perumalsamy et al., 2009; Chu et al., 2011; Zahran and Abdelgaleil, 2011; Liu et al., 2013; Yildirim et al., 2013; Benelli et al., 2018a, 2018b; Zhang et al., 2019). Essential oils containing ß-eudesmol, bornyl acetate and camphene as major constituents have shown strong activity (Park et al., 2003; Yang et al., 2004;

Chu et al., 2011; Liu et al. 2013, 2014a; Chen et al., 2018; Benelli et al., 2018; Mothana et al., 2018; Huong et al., 2020; Feng et al., 2019, 2020). Beta-eudesmol showed an 80% mortality when administered at rate of exposure 78.6 $\mu g/cm^2$ against *Ae. aegypti* (Paluch et al., 2009). It was also tested for larvicidal effects against *Culex pipiens pallens* larvae with mortality of 90 and 95% at the concentrations of 0.05 and 0.1 mg/mL, respectively, with a loss of efficacy at lower concentrations (Park and Park, 2012). The essential oil of *Cirsium pendulum* Fisch., containing ß-eudesmol as major compound, exhibited a larvicidal effect against *Ae. albopictus* (LD_{95}: 143.54 μg/mL) (Liu et al., 2014b). The LC_{50} value of eudesmol was determined as 2.25 ppm and the combination of eudesmol and malathion were found to be one of the most active combinations (Sarma et al., 2019). Perumalsamy et al. found camphene, from the essential oil of *Asarum heterotropoides* roots, had larvicidal activity (LC_{50}: 67.02 ppm) against early third-instar larvae of *Ae. aegypti* 24 h after treatment (Perumalsamy et al., 2009). Contrary to this, (-)-camphene and (+)-camphene exhibited activity with the LC_{50} values of 220 and 406 ppm, respectively, whereas *p*-cymene showed the highest larvicidal effect (LC_{50}: 51 ppm). It was also deduced that the lipophilicity correlated with the larvicidal activity (Santos et al., 2010). After two years, it was shown that, (-)-camphene and (+)-camphene had a larvicidal activity 77.5% and 100%, respectively, at the concentration of 0.1 mg/mL (Seo et al., 2012). Similar to these studies, both nonpolar extracts we examined exhibited significant adulticidal activity against *Ae. aegypti*. However, CM1 showed higher mortality (almost 100%) than HM1. This suggests that compounds with moderate polarity in this plant are more active than compounds with low polarity.

4. Conclusion

This is the first study examining cytotoxicity, against HepG2 and HUVEC cell lines, and the insecticidal activity of *V. dioscoridis*. Considering the results of the testing, the extracts of *V. dioscoridis* have good potential as possible cytotoxins or insecticides. Also, we observed that compounds with different polarities had differing cytotoxic and insect repellent activities. Better cytotoxicity was obtained from polar preparations of *V. dioscoridis*. The insecticidal activity determined that the extracts with moderate polarity such as chloroform extract have more activity against *Ae. aegypti* and other insects. Previous investigations on the activities of major compounds found in *V. dioscoridis* essential oil, like ß-eudesmol, bornyl acetate, camphene and maaliol showed the compounds can have cytotoxic activity, insecticidal activity or both. This warrants further studies on this potential of *V. dioscoridis* to determine the potential for development of commercially useful agents.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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