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Isolation of eudesmane type sesquiterpene ketone from *Prangos heyniae* H.Duman & M.F.Watson essential oil and mosquitocidal activity of the essential oils

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Abstract: In the present work, an endemic species *Prangos heyniae* collected in four locations from Turkey was subjected to hydrodistillation in Clevenger type apparatus to obtain the essential oils (EO1-4). The gas-chromatography/ mass spectrometry (GC/MS) and gas-chromatography-flame ionization detector (GC/FID) analyses showed that the EOs were rich in sesquiterpenes, germacrene D (10.3-12.1%), β-bisabolene (14.4%), kessane (26.9%), germacrene B (8.2%), elemol (3.4-46.9%), β-bisabolenal (1.4-70.7%), β-bisabolenol (8.4%) and an eudesmane type sesquiterpene (**1**) (16.1%) with [M$^+$218]. This unidentified compound (**1**) was isolated in a rapid one-step manner with >95.0% purity using Preparative Capillary Gas Chromatography (PCGC) with an HP Innowax column connected to a Preparative Fraction Collector (PFC) system. Structure determination was accomplished from 1D- and 2D-NMR spectroscopic data which determined a new eudesmane type sesquiterpene, 3,7(11)-eudesmadien-2-one (**1**). Using a biting deterrent bioassay, the mean proportion not biting (PNB) values of the *P. heyniae* EO1-4 were 0.88 for EO1 and 0.80 for EO2 which were similar to the positive control DEET (*N*, *N*-diethyl-3-methylbenzamide). The EO3 and EO4 had lower PNB values of 0.64 and 0.44, respectively. *P. heyniae* EO1-4 showed good larvicidal activity at 125 and 62.5 ppm whereas EO1-3 were slightly less effective at the dose of 31.25 ppm and EO4 was not active at 31.25 ppm against 1st instar *Aedes aegypti*.

Keywords: *Prangos heyniae*, 3,7(11)-eudesmadien-2-one, preparative gas chromatography, NMR, *Aedes aegypti,* larvicidal and biting deterrent

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1 Introduction

Prangos Lindl. is one of the important genera of Umbelliferae in the world and the largest genus after *Ferula* L., *Bupleurum* L., *Pimpinella* L., *Heracleum* L., *Seseli* L., *Angelica* L., and *Bunium* L. [1]. The genus *Prangos* encompasses *ca*. 43 species mainly distributed in the Asian hemisphere and 13 species are recorded for Turkey [2]. Some representatives of the genus are consumed as a food additive or as an infusion for their organoleptic properties [2]. In traditional medicine of Turkey, *Prangos* species are used as wound healing and stimulant for gastric problems [3]. An ethnopharmacological study on *Prangos* species has recently been reported by Bulut et al. [4]. The local name used for *Prangos* species is "çakşir" where the name is given to various species of Apiaceae genera like *Prangos*, *Ferulago*, *Ferula* and *Peucedanum* in Turkey. These genera are used as an aphrodisiac or for fertility both in humans and in animals in southern part

Table 1: Ethnopharmacological data on the *Prangos* species (literature survey).

Prangos species	Plant part	Therapeutic effect	Procedure	Adm.#	Ref.
P. asperula Boiss.	Aerial part	Reduce blood pressure, skin disease, digestive disorder, hemorrhoid	Infusion	Int.*	[6]
P. ferulacea (L.) Lindl.	Root	Aphrodisiac	Grated (+ honey)	Eaten	[4, 7]
	Young shoots	Diabetes,	Boiled	Int.	
	Leaves	Antihypertensive	Infusion	Int.	
	Aerial parts	Aphrodisiac	Decoction	Int.	
P. meliocarpoides Boiss.	Root	Aphrodisiac	Planed (+ honey)	Eaten	[7]
P. pabularia Lindl.	Root	Aphrodisiac	Planed (+ honey)	Eaten	[3]
	Root	Wound	Crushed	Ext.**	
	Leaves	Dyspepsia	Decoction	Int.	
P. platychlaena Boiss.	Root	Wound (animal)	Dried then crushed	Ext.	[8]
	Root	Aphrodisiac	Planed (+ honey)	Int.	
	Root	Intestinal diseases	Gum	Int.	
		Stop bleeding		Ext.	
P. uechtritzii Boiss. et Hausskn.	Whole plant	Hemorrhoids	Boiled in vinegar	Ext.	[4]

* internal; ** external; # admitting.

of Turkey [5]. The ethnopharmacological study on *Prangos* species are summarized in **Table 1** [3, 4, 6-8].

Previous phytochemical investigations with *Prangos* species determined mono-, sesqui- and diterpenes [9], coumarins [10], phenols, phenolic acids, flavonoids [11] and carotenoids [12]. A number of species were subjected to investigation for EOs. The main constituents detected in *Prangos* essential oils (EOs) are summarized in **Table 2** [13-27]. As can be seen from **Table 2**, essential oils obtained different *Prangos* species contain a wide range of diverse compounds.

To date, a wide range of biological activities of *Prangos* species are scientifically approved: antioxidant [28-30], antibacterial [9, 31], antiviral [32, 33], antiproliferative [34], phytotoxic, cytotoxic and apoptogenic [33, 35, 36], antidiabetic [37], hypoglycemic [38], insecticidal [39], antimosquito [40], antispasmodic [41], analgesic [42]. *P. ferulacea* seems to be the most investigated species for different biological activities [18, 28, 29, 31, 37, 41, 43-48]
In the present work, we tried to perform a comprehensive investigation of *P. heyniae* for essential oil chemical composition and isolation of target constituent as well as evaluation of larvicidal activity against 1st instar *Aedes aegypti* L. The isolation of the pure compound can be (i) time-consuming and tedious using classical preparative chromatographic procedures for EOs; (ii) complicated by the risk of the compounds being lost and (iii) altered, or contaminated during isolation process. To overcome these difficulties, the combination of PCGC-PFC with GC/MS and GC-FD was applied. The combination of PCGC and PFC was reported to have the following advantages: efficient harvesting of individual compounds as well as mixtures of several compounds, accelerated separation experiment, simultaneous and multiple separations of several constituents from the complex mixture [49-51]. Previous studies demonstrated that this technique allowed the harvesting of pure individual constituents in microgram level from the complex mixtures like alkanes and fatty acids from archaeological samples and polycyclic aromatic hydrocarbons from environmental materials [52] as well as individual enantiomers [53-55].

2 Experimental

2.1 General

All organic solvents and reagents used for PCGC were of analytical or chromatographic grade. Anhydrous sodium sulfate (ACS-ISO, for analysis), *n*-hexane (ACS, for analysis) was purchased from Carlo Erba (Milan, Italy). Optical rotation was recorded on a Rudolph Research Analytical Autopol IV automatic polarimeter (Rudolph, Hackettstown, NJ, USA). 1D- and 2D-NMR spectra were obtained on a Bruker Inova 600 MHz spectrometer (Bruker BioSpin Corp., Billerica, MA, USA). IR spectra were recorded on Agilent Technologies Cary 630 FTIR spectrometer (Santa Clara, CA, USA). High resolution mass spectra were measured on Agilent Technologies (Model #G1969A, Santa Clara, CA, USA).

Table 2: The main compounds detected in EOs of *Prangos* species (literature survey).

Prangos species	Plant part	Main constituents (%)	Ref.
P. acaulis (DC) Bornm.	AP	α-pinene (13.6), limonene (12.9), myrcene (8.1), β-pinene(5.4) δ-3-carene(25.5), α-terpinolene(14.7) caryophyllene (2.9), γ-curcumene (2.6)	[13]
	AP	*cis*-sesquisabinene hydrate (25.6), α-pinene (12.5)	[14]
P. asperula Boiss.	Fr	sabinene (20.6), β-phellandrene (19.0), γ-terpinene (9.0)	[15]
P. coryombosa Boiss.	AP	β-elemene (22.0), spathulenol (12.5), kessane (10.7)	[16]
P. denticulate Fisch & Mey	Fr	sabinene (26.1), *p*-cymene (19.7)	[17]
	R	δ-3-carene (49.3), (Z)-3,5-nonadiyne-7-ene (20.4)	
P. ferulaceae (L.) Lindl	AP	β-phellandrene (20.3), α–terpinolene (15.2), α-pinene (11.5), δ-3-carene (11.0), α-phellandrene (9.0), *trans*-β-ocimene (9.6)	[18]
P. heyniae H.Duman & M.F.Watson	Fr	β-bisabolenal (53.3 and 18.0), β-bisabolenol (14.6 and 2.3) and β-bisabolene (12.1 and 10.1), germacrene D (13.5) and germacrene B (9.4)	[19]
P. latiloba Korovin	Fl	limonene (18.3), myrcene (10.4), (*E*)-β-ocimene (7.8), α-phellandrene (6.4) and α-pinene (5.7)	[20]
	L	limonene (17.4), myrcene (9.4), α-pinene (6.1), α-phellandrene (5.4) and (*E*)-β-ocimene (5.3)	
	S	limonene (13.5), myrcene (8.6), α-phellandrene (4.9), germacrene-D (4.5) and γ-curcumene (4.3)	
P. pabularia Lindl.	Fr	α-humulene (16.6 and 15.5), bicyclogermacrene (16.1 and 7.9), spathulenol (10.6 and 5.7), germacrene D (5.7 and 2.9) and α-pinene (4.2 and 23.9)	[21]
	L	spathulenol (16.0), α-bisabolol (14.3)	[22]
P. peucedanifolia Fenzl	Fl	β-pinene (35.5), α–pinene (22.1), β-phellandrene (12.5)	[9]
	L	*m*-cresol (50.3)	
P. platychlaena Boiss.		δ-3-Carene (3.3), *p*-cymene (3.3)	[23]
P. scabra Nabelek	Fr	β-elemene (23.3), (*Z*)-β-farnesene (16.2), epi-globulol (1.5), γ-cadinene (10.0), β-caryophyllene (9.2)	[24]
	Fl	epi-globulol (21.9), β-elemene (19.7), caryophyllene oxide (9.0), α-cadinol (6.2)	
P. serpentinica (Rech.f., Aell. Esfand.)	AP	β–carryophyllene (26.4), δ-3-carene (6.1), linalool (5.7), α-phellandrene (5.3), *p*-cymene (5.2), camphene (5.1), α-pinene (3.7)	[25]
P. uechtritzii Boiss. & Hausskn.		α-pinene (40.8), nonene (17.0), β-phellandrene (11.1), δ-3-carene (7.3), p-cymene (4.9)	[23]
	AP	*p*-cymene (10.9), γ-terpinene (7.0), β–phellandrene (7.8), α-phellandrene (6.3)	[26]
P. uloptera DC.	AP	β-caryophyllene (27.1), caryophyllene oxide (15.9), α-pinene (12.4)	
	Fl	saferole (21.6), α–pinene (20)	[27]
	Fl	α-bisabolol (30.5), saferole (19.1), (+)-spathulenol (12.9)	
	Fr	α-terpinene (35.5), *trans*-anethole (23.5)	

AP: Aerial part; Fr: fruit; R: root; Fl: flower; L: leaf; S: stem.

2.2 Plant material

The collection data of four *P. heyniae* samples, voucher specimen code, EO yields are presented in **Table 3**. The identity was confirmed by anatomical examination in comparison with the herbarium specimen retained in the Herbarium of Selcuk University. The plant material was identified by A. Duran.

2.3 Essential oil isolation

The EO was isolated by hydrodistillation for 3 hours of the dried aerial parts of *P. heyniae*. The EO was dried over anhydrous sodium sulfate and stored in sealed vials in a refrigerator (4°C), until GC/FID and GC/MS analyses and biological activity testing. The EO was dissolved in *n*-hexane (10%, v/v) to conduct chromatographic determination of their composition. The oil yield was calculated on dry weight basis (**Table 3**).

Table 3: *Prangos heyniae* samples collection data and the oil yields.

P. heyniae sample	Voucher specimen code	Collection place	Oil yield, %
EO1-herb oil	AD 7876	Konya: Bozkir-Korualan road, 37 01 186 N, 32 20 008 E, Korualan 1.km	0.5
EO2-fruit oil	AD 8059	Konya: Hadim-Taşkent road	0.9
EO3-herb oil	AD 7879	Konya: Korualan-Hadim road, 36 59 952 N, 032 20 716 E	0.4
EO4-fruit oil	AD 8055	Konya: Bozkir-Hadim road, 37 01 138 N, 32 20 011 E	0.3

2.4 GC/MS analysis

At the first stage of the experiment, the oil was analyzed by GC/FID and GC/MS techniques. GC/MS analysis was carried out with an Agilent 5975 GC-MSD system (Agilent, USA; SEM Ltd., Istanbul, Turkey). HP-Innowax FSC column (60 m × 0.25 mm, 0.25 µm film thickness, Agilent, USA) was used with a helium carrier gas at 0.8 mL/min. GC oven temperature was kept at 60°C for 10 minutes and programmed to 220°C at a rate of 4°C /min, kept constant for 10 minutes at 220°C, and then programmed to increase at a rate of 1°C/min to 240°C. The oil was analyzed with a split ratio of 40:1. The injector temperature was 250°C. Mass spectra were taken at 70 eV and the mass range was from m/z 35 to 450.

2.5 GC/FID analysis

The GC/FID analysis was carried out with capillary GC using an Agilent 6890N GC system (SEM Ltd., Istanbul, Turkey). Flame ionization detector (FID) temperature was set at 300°C in order to obtain the same elution order with GC/MS. Simultaneous injection was performed using the same column and appropriate operational conditions like in GC/MS.

2.6 Essential oil fractionation and isolation of target compound 1 with PC-GC system

At the second stage of the experiment, the oils with unknown constituents (target compound) were subjected to fractionation in order to isolate and concentrate the target compound using PCGC connected to PFC.

2.6.1 PCGC System

Briefly, the oil containing the compound of interest was repeatedly injected by an autosampler into GC equipped with a cooled injection system (CIS) and preparative capillary column. The end of the column was connected to a zero dead volume effluent splitter, which diverted a portion (1.0 %) of the effluent to the detector while the majority (99.0%) was transferred to and selected fraction was trapped using a preparative fraction collector (PFC) unit. Trapping of the isolated compound in collector vials (cooled with N_2) during the course of multiple injections has resulted with enough quantity (yield was 0.2 mg from each 3 µL of the oil) to facilitate its subsequent identification with different spectroscopic techniques.

PCGC system consisted of an Agilent 7890 GC (Agilent, USA; SEM Ltd., Istanbul, Turkey), equipped with a flame ionization detector (FID) and 5975 MSD with Triple-Axis Detector, Agilent G 4513 autoinjector, integrated with the CIS (Gerstel, Germany; SEM Ltd., Istanbul, Turkey), a zero-dead-volume effluent splitter, and a preparative trapping device. The preparative device consists of seven-port zero-dead-volume valve in a heated interface (≈300°C) and seven 200 µL glass U-tube traps (six sample traps and one waste trap) supported in liquid nitrogen-cooled (-30°C) units. The Gerstel multicolumn switching system has been used. The autoinjector, CIS, and trapping device are programmable and controlled by Gerstel Modular Analytical Systems (MAS) using Maestro software.

2.6.2 Conditions of PCGC procedure

The compound of interest was isolated from the oil using a HP Innowax (30 m × 0.53 mm × 1.0 µm film thickness, USA) preparative capillary column with helium carrier gas (flow rate 6 mL/min, average flow rate 46.843 cm/sec). GC oven temperature was kept 90°C for 0 minutes then 15°C/min to 195°C for 0 minutes, then 60°C/min to 230°C for 2.4167 minutes. The total time was 10 minutes. The oil was analyzed in splitless mode. The injector temperature was 250°C. Mass spectra were taken at 70 eV and the mass range was from m/z 35 to 450. PFC transfer and PFC distribution temperatures were kept at 220°C and 230°C, respectively. PFC trap cooled with

liquid nitrogen was kept at -30°C. Retention time interval (cut time) was estimated between 18.40 min and 19.00 min for the target constituent. Injector volume was 4 µL. After PCGC isolation, the U-tube containing the trap with isolated compound was detached, and the content was recovered by addition of hexane (0.5 mL) and transferred to 2-mL glass vials. An aliquot (50.0 µL) was removed and transferred to the GC/FID and GC-MS autosampler vial for determination of purity and yield.

2.7 Identification and quantification of compounds

Compounds were identified by comparison of the chromatographic peaks retention times with those of authentic compounds analyzed under the same conditions, and by comparison of the retention indices (as Kovats indices) with literature data. Comparisons of MS fragmentation patterns with those of standards and mass spectrum database search were performed using the Wiley GC-MS Library (Wiley, New York, NY, USA), MassFinder software 4.0 (Dr. Hochmuth Scientific Consulting, Hamburg) [56], Adams Library [57], and NIST Library. Confirmation was also achieved using the in-house "Başer Library of Essential Oil Constituents" database, obtained from chromatographic runs of pure compounds performed with the same equipment and conditions. A C_9–C_{40} *n*-alkane standard solution (Fluka, Buchs, Switzerland) was used to spike the samples for the determination of chromatographic retention indices (RI). Percent composition was obtained for each constituent on the basis of GC/FID analysis of the oil.

2.8 Mosquito bioassays

2.8.1 Mosquitoes

Aedes aegypti larvae used in these studies were from a laboratory colony maintained at the Mosquito and Fly Research Unit at the Center for Medical, Agricultural and Veterinary Entomology, USDA-ARS, Gainesville, Florida using standard rearing practices [58]. For biting deterrence bioassays, pupae were maintained in the laboratory at 27 ± 2° C and 60 ± 10% RH in a photoperiod regimen of 12:12 h (L:D). For larvicidal bioassays, the eggs were hatched and the larvae were maintained at the above temperature.

2.8.2 Mosquito biting bioassays

Experiments were conducted by using a six-celled *in vitro* Klun and Debboun (K&D) module bioassay system developed by Klun et al. [59] for quantitative evaluation of biting deterrent properties of candidate compounds. Briefly, the assay system consists of a six-well reservoir with each of the 3 x 4 cm wells containing 6 mL of blood. As described by Ali et al.[58], a feeding solution consisting of CPDA-1 and ATP was used instead of blood. Green fluorescent tracer dye (www.blacklightworld.com) was used to determine the feeding by the females. Treatments of *P. heyniae* EOs were applied at 10 µg/cm^2, and DEET (97%, *N*, *N*-diethyl-3-methylbenzamide, Sigma Aldrich, St. Louis, MO) at 25 nmol/cm^2 was used as positive control. All the treatments were freshly prepared in molecular biology grade 100% ethanol (Fisher Scientific Chemical Co. Fairlawn, NJ) at the time of bioassay. A replicate consisted of six treatments: four test compounds, DEET and ethanol treated organdy as solvent control. A set of 5 replications each with 5 females per treatment were conducted using a newly treated organdy and a new batch of females in each replication.

2.8.3 Larvicidal bioassays

Bioassays were conducted to test samples of the essential oil of *P. heyniae* for their larvicidal activity against *Ae. aegypti* by using the bioassay system described by Pridgeon et al. [60]. First, instar *Ae. aegypti* larvae were added in a droplet of water to each well of 24-well plates (BD Labware, Franklin Lakes, NJ) by using of a disposable 22.5 cm pasteur pipette. Fifty microliters of larval diet (2% slurry of 3:2 Beef Liver powder (Now Foods, Bloomingdale, Illinois) and Brewer's yeast (Lewis Laboratories Ltd., Westport, CT) was added to each well by using a Finnpipette stepper (Thermo Fisher, Vantaa, Finland). All samples tested were diluted in DMSO (Dimethyl sulfoxide, Sigma Aldrich, St. Louis, MO). Eleven microliters of the test chemical was added to the labeled wells, while 11 µL of ethanol was added to control treatments. After the treatment application, the plates were swirled in clockwise and counterclockwise motions and front and back and side to side five times to ensure even mixing of the chemicals. Larval mortality was recorded 24-hours post treatment. Larvae that showed no movement in the well after manual disturbance of water were recorded as dead. Permethrin (Chemservice, Westchester, PA, USA) was used as positive control and DMSO as negative control.

2.9 Statistical analyses

Proportion not biting (PNB) was calculated using the following formula:

$$\text{PNB} = 1 - \left(\frac{\text{Total no. of biting females}}{\text{Total no. of females}}\right)$$

Data on the PNB values were analyzed using the ANOVA procedure of SAS (version 9.2; SAS Institute Inc., Cary, NC). Larvicidal mortality is presented as percentage.
Ethical approval: The conducted research is not related to either human or animals use.

3 Results and Discussion

3.1 Chemical composition

Our work aimed to elucidate further knowledge on the secondary metabolites of *Prangos* species from Turkey and search for the novel biological properties for these species. The GC/FID and GC/MS techniques allow us to determine qualitative and quantitative profile of four *P. heyniae* EOs. The list of detected compounds with their relative percentages, retention indices and method of identification is given in **Table 4** in order of their elution on the HP-Innowax FSC column. GC analysis of EO1, EO2, EO3 and EO4 resulted with 66, 20, 56 and 61 constituents representing 96.7%, 97.9%, 97.4% and 96.3% of the oils, respectively. The EOs were characterized with high diversity of volatile constituents, which were classified as mono- and sesquiterpene hydrocarbons and their oxygenated forms.

Oxygenated sesquiterpenes (68.5 - 80.3%) were the most abundant groups in EO1-3 with the exception of EO4 in which sesquiterpene hydrocarbons (60.0%) predominated. The monoterpenes were presented in scarce amount (0.1 - 6.7%) in all the EOs. EO1 was found to be rich with elemol (29.5%), germacrene D (12.1%) and unidentified constituent (**1**) with M^+218 were found to be the main constituents of EO. It seems to be different from two *P. heyniae* EOs reported previously [61]. In the EO2, β-bisabolenal (70.7%), β-bisabolene (14.4%) and β-bisabolenol (8.4%) were found to be the main compounds. It seems to be close to the oil composition reported by Baser, in which β-bisabolenal (53.3% and 18.0%), β-bisabolenol (14.6% and 2.3%) and β-bisabolene (12.1% and 10.1%) were detected as the main constituents. Approximately half of EO3 was elemol (46.9%),however, germacrene D (11.9%) was also detected. The fourth oil sample (EO4) was distinguished with kessane (26.9%) and germacrene B (8.2%), although it contained germacrene D (10.3%).

In our work, the preliminary GC/FID and GC/MS analyses of *P. heyniae* EO1 revealed a presence of an unidentified constituent (**1**) with M^+218. The chromatographic profile of EO1 obtained on analytical polar column Innowax is presented in **Figure 1.** The fractionation of EO1 and isolation of **1** was performed using PCGC connected with PFC. PCGC has generated far superior purity: the targeted constituent **1** with relative retention indice RRI=2601 was isolated from the *P. heyniae* EO with >95.0% purity (**Figure 2**). The selected region with the target compound (between 18.40 min and 19.00 min) is shown shaded on the chromatogram obtained on preparative column (**Figure 2**). The target peak was trapped into the sample microcollector (U-shaped trap), while the rest of the peaks were trapped into the waste microcollector. The sample microcollector was cooled by liquid nitrogen (-30°C) to prevent the trapped constituent from evaporating during the isolation procedure. Fraction recovery was 0.4 mg/inj. Trapping of the isolated compound **1** with an external cryotrap collection device during the course of multiple injections produced a sufficient quantity to facilitate subsequent NMR spectroscopic analysis as well as mass spectrometry. In the present work, the target constituent was isolated from the oil by using the tandem of the automated PCGC and PFC, which allowed separating and recovering of sufficient quantities of individual compound with high purity. The separation was completed quickly from the complex oil matrix with minimal prior fractionation.

Structure determination of the isolated constituent **1** was accomplished from 1D- and 2D-NMR spectroscopic data. HR-ESI-MS analysis of **1** suggested a molecular formula of $C_{15}H_{22}O$ (*m/z* 219.1743 $[M+H]^+$) (Calculated for $C_{15}H_{22}O$, 218.1671). ^{13}C, DEPT-135 and HSQC spectra of **1** revealed one ketone carbonyl (δ_C 198.9), four quaternary carbons (including three olefinic at δ_C 163.0, 129.5 and 123.0), one olefinic methine carbon (δ_C 126.9), four methylene groups (δ_C 54.3, 40.6, 27.0 and 24.7), one up-field methine carbon (δ_C 48.3) and four tertiary methyl groups (δ_C 21.8, 20.2, 20.1 and 16.3). The presence of 15 carbon resonances together with an overall spectral profile and MS data (Unsaturation Number=5; olefinic system=3) suggested a bicyclic sesquiterpene structure (**Figure 3**). In the ^1H-NMR spectrum, three of the methyl groups resonated in the lower field (1.67, 1.69 and 1.92) indicating their localization on double bond carbons. Moreover, low-field shift of one of the olefinic carbons at δ_C 163.0 suggested an α,β-

Table 4: Chemical composition of *Prangos heyniae* essential oils.

	RRI[#]	RRI[§]	Compound	EO1	EO2	EO3	EO4	ID method
1.		1032 [73]	α-Pinene	0.2	1.5	0.3	4.1	a,b,c
2.		1035 [73]	α-Thujene	t				a,b,c
3.		1076 [73]	Camphene		t	t	0.4	a,b,c
4.		1118 [73]	β-Pinene	t	t	t	0.5	a,b,c
5.		1132 [73]	Sabinene	t		t	t	a,b,c
6.		1161 [74]	Myrcene	0.1	t	t	t	a,b,c
7.		1186 [75]	α-Phellandrene			t		a,b,c
8.			Dehydro-1,8-cineole	0.4		0.6	t	b,c
9.		1203 [73]	Limonene	t	0.8	t	1.2	a,b,c
10.		1207 [76]	β-Phellandrene	t		t	t	a,b,c
11.		1242 [77]	Pentyl furan	t		t		b,c
12.		1234 [78]	(Z)-β-Ocimene			t	0.5	a,b,c
13.		1256 [79]	γ-Terpinene	t		t		a,b,c
14.		1250 [78]	(*E*)-β-Ocimene	t		t		a,b,c
15.		1278 [79]	*p*-Cymene	t		t	t	a,b,c
16.		1448 [80]	Hexyl-3-methyl butyrate				0.2	a,b,c
17.		1480 [75]	α-Cubebene	t		t	t	a,b,c
18.		1468 [78]	δ-Elemene	0.1				a,b,c
19.		1493 [81]	α-Ylangene	t				a,b,c
20.		1497 [73]	α-Copaene	0.2		0.2	0.4	a,b,c
21.			α-Bourbonene	t			t	a,b,c
22.		1535 [79]	β-Bourbonene	0.1		t	1.0	a,b,c
23.		1547 [81]	β-Cubebene	0.1		t	t	a,b,c
24.		1576 [82]	β-Ylangene	0.2		t		a,b,c
25.		1585 [82]	β-Elemene	0.8		0.5	1.7	a,b,c
26.		1587 [83]	β-Copaene	t		t	t	a,b,c
27.		1611 [84]	β-Caryophyllene	2.3	0.2	2.4	1.5	a,b,c
28.			6,9-Guaiadiene	t				a,b,c
29.		1650 [81]	γ-Elemene	0.2			2.6	a,b,c
30.		1663 [85]	Alloaromadendrene	t		t	0.2	a,b,c
31.		1666 [78]	(*Z*)-β-Farnesene		0.2			a,b,c
32.		1688 [86]	*epi*-Zonarene				t	a,b,c
33.		1687 [73]	α-Humulene	0.8	t	0.8	1.4	a,b,c
34.		1704 [73]	γ-Muurolene	0.2		0.1	t	a,b,c
35.		1697 [73]	Guaioxide				2.3	b,c
36.		1726 [81]	Germacrene D	**12.1**	t	**11.9**	**10.3**	a,b,c
37.		1740 [81]	α-Muurolene			0.4	t	b,c
38.		1737 [74]	β-Bisabolene		**14.4**			a,b,c
39.		1738 [75]	β-Selinene	2.3				a,b,c
40.		1735 [75]	α-Selinene	0.6				a,b,c
41.		1756 [81]	Bicyclogermacrene	0.6		0.7	1.1	a,b,c
42.		1770 [82]	Citronellol		t			a,b,c
43.		1773 [81]	δ-Cadinene	0.7	t	1.5	3.3	a,b,c
44.		1776 [81]	γ-Cadinene			0.1	1.4	a,b,c

Continued **Table 4:** Chemical composition of *Prangos heyniae* essential oils.

	RRI[#]	RRI[§]	Compound	EO1	EO2	EO3	EO4	ID method
45.		1785 [73]	Kessane		t		**26.9**	b,c
46.		1790 [87]	Selina-3,7(11)-diene	4.0				a,b,c
47.		1856 [79]	Germacrene-B	0.5			**8.2**	a,b,c
48.		1899 [86]	*epi*-Cubebol	t				a,b,c
49.		1941 [73]	α-Calacorene	t				a,b,c
50.		1912 [88]	1,5-Epoxy-salvial(4)14-ene	0.7		0.7	0.6	a,b,c
51.			Elema-1,3-dien-6-alpha-ol (= 6-epi-shyobunol)	0.2				a,b,c
52.		1929 [86]	Cubebol	0.2		0.2	t	a,b,c
53.			1-*endo*-Bourbonanol			t	t	b,c
54.		2001 [73]	Isocaryophyllene oxide	0.2		0.1	t	a,b,c
55.		2008 [89]	Caryophyllene oxide	2.0	0.3	1.9	2.5	a,b,c
56.			1-Methyl-4-(5-methyl-1-methylene-hex-4-enyl)-7-oxabicyclo [4.1.0] heptane (= Aurean)		0.5			c
57.			2,3,6-Trimethylbenzaldehyde			t		a,b,c
58.		2037 [73]	Salvial-4(14)-en-1-one	1.0		0.2	0.5	a,b,c
59.			10-epi-Elemol			0.9		a,b,c
60.		2050 [79]	(*E*)-Nerolidol		t			a,b,c
61.		2060 [84]	Ledol			0.2	0.5	a,b,c
62.			1,6-Germacradien-5β-ol	1.0		2.0	2.3	a,b,c
63.		2080 [85]	Cubenol				t	a,b,c
64.			1,10-Di-*epi*-Cubenol				0.2	b,c
65.		2098 [81]	β-Oplopenone				t	a,b,c
66.		2096 [73]	Elemol	**29.5**		**46.9**	3.4	a,b,c
67.		2108[81]	α-Guaiol			t		a,b,c
68.		2093 [75]	Viridiflorol				0.8	a,b,c
69.			Hedycaryol	0.2				a,b,c
70.		2130 [90]	Salviadienol				0.5	a,b,c
71.		2136 [82]	Spathulenol	0.7		0.9	1.7	a,b,c
72.			Nor-copaanone				0.4	a,b,c
73.		2177 [75]	γ-Eudesmol	2.1		4.8	1.7	a,b,c
74.		2185 [79]	T-Cadinol				t	a,b,c
75.		2187 [91]	Thymol			0.9		a,b,c
76.			1,6-Germacradien-5α-ol	0.8				a,b,c
77.		2178 [92]	Eremoligenol	t		0.5		b,c
78.		2209 [79]	T-Muurolol	0.6		1.4	1.7	a,b,c
79.		2246 [78]	Torreyol			t	0.6	b,c
80.		2229 [82]	α-Eudesmol	2.5		4.1	0.9	a,b,c
81.		2218 [82]	α-Cadinol	2.1		5.5	4.6	a,b,c
82.			*cis*-Guai-9-en-11-ol				0.5	c
83.		2258 [73]	β-Eudesmol	1.2		1.1		a,b,c
84.			Porosadienol	1.3				a,b,c
85.			Guaia-6,10(14)-dien-4-β-ol			0.6		a,b,c
86.			Eudesma-4(15),7-dien-1-ol isomer	0.9		0.7	0.5	c

Continued **Table 4:** Chemical composition of *Prangos heyniae* essential oils.

RRI#	RRI§	Compound	EO1	EO2	EO3	EO4	ID method
87.	2324 [73]	Caryophylla-2(12),6(13)-dien-5α-ol (=*Caryophylladienol II*)			0.5		a,b,c
88.		Eudesma-4(15),7-dien-1-β-ol	0.7		0.6	0.6	a,b,c
89.		β-Bisabolenal	2.5	**70.7**	4.1	1.4	a,b,c
90.	2392 [73]	Caryophylla-2(12),6-dien-5β-ol (=*Caryophyllenol II*)	0.4	0.9		1.2	a,b,c
91.		(4aR,6R,8aR)-6-Isopropenyl-4,8a-dimethyl-4a,5,6,7,8,8a-hexahydro-2(1H)-naphthalenone*	1.6				c
92.	2500 [73]	Pentacosane	0.3				a,b,c
93.		β-Bisabolenol		**8.4**			a,b,c
94.		3,7(11)-Eudesmadien-2-one (**1**)¥	**16.1**				d
95.		14-Hydroxy-δ-cadinene	t		t	t	a,b,c
96.	2606 [78]	Phytol	0.3				a,b,c
97.	2700 [81]	Heptacosane	0.2				a,b,c
98.	2931 [73]	Hexadecanoic acid	0.9	t	t	t	a,b,c
		Total	**96.7**	**97.9**	**97.4**	**96.3**	
Monoterpene hydrocarbons			0.3	2.3	0.3	6.7	
Oxygenated monoterpenes			0.4	t	1.5	t	
Sesquiterpene hydrocarbons			25.8	14.8	18.6	60.0	
Oxygenated sesquiterpenes			68.5	80.3	76.9	27.1	
Others			1.7	0.5	0.1	2.5	

RRI# Relative retention indices calculated against *n*-alkanes (C_9-C_{40}) on HP-Innowax column; RRI§ Relative retention indices reported in literature; % calculated from FID data; tr Trace (< 0.1 %); ¥GC-MS m/z (rel. int.): 218 (100), 175 (78), 161 (50), 147 (40), 135 (38), 119 (44), 105 (34), 91 (44), 79 (30), 67 (26), 55 (21), 41 (32); * Correct isomer not identified; a) Identification based on retention index of genuine compounds on the HP-Innowax column; b) Identification on the basis of computer matching of the mass spectra and retention times from Başer Library, c) Identification on the basis of computer matching of the mass spectra from Adams, MassFinder, Wiley, NIST libraries; d) Identification on the basis of NMR spectra.

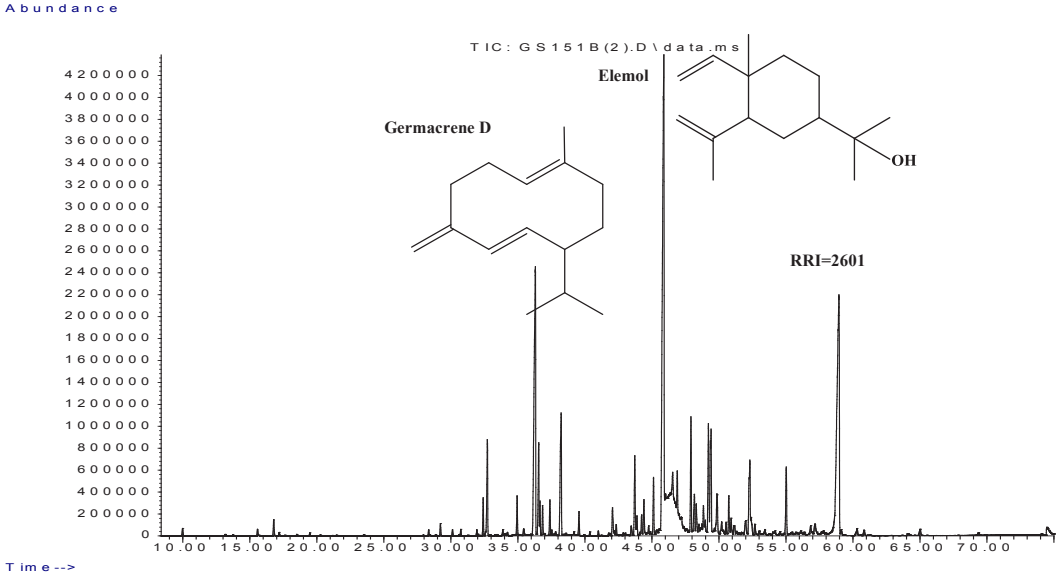

Figure 1: Chromatographic profile of *P. heyniae* EO on analytical polar HP Innowax column.

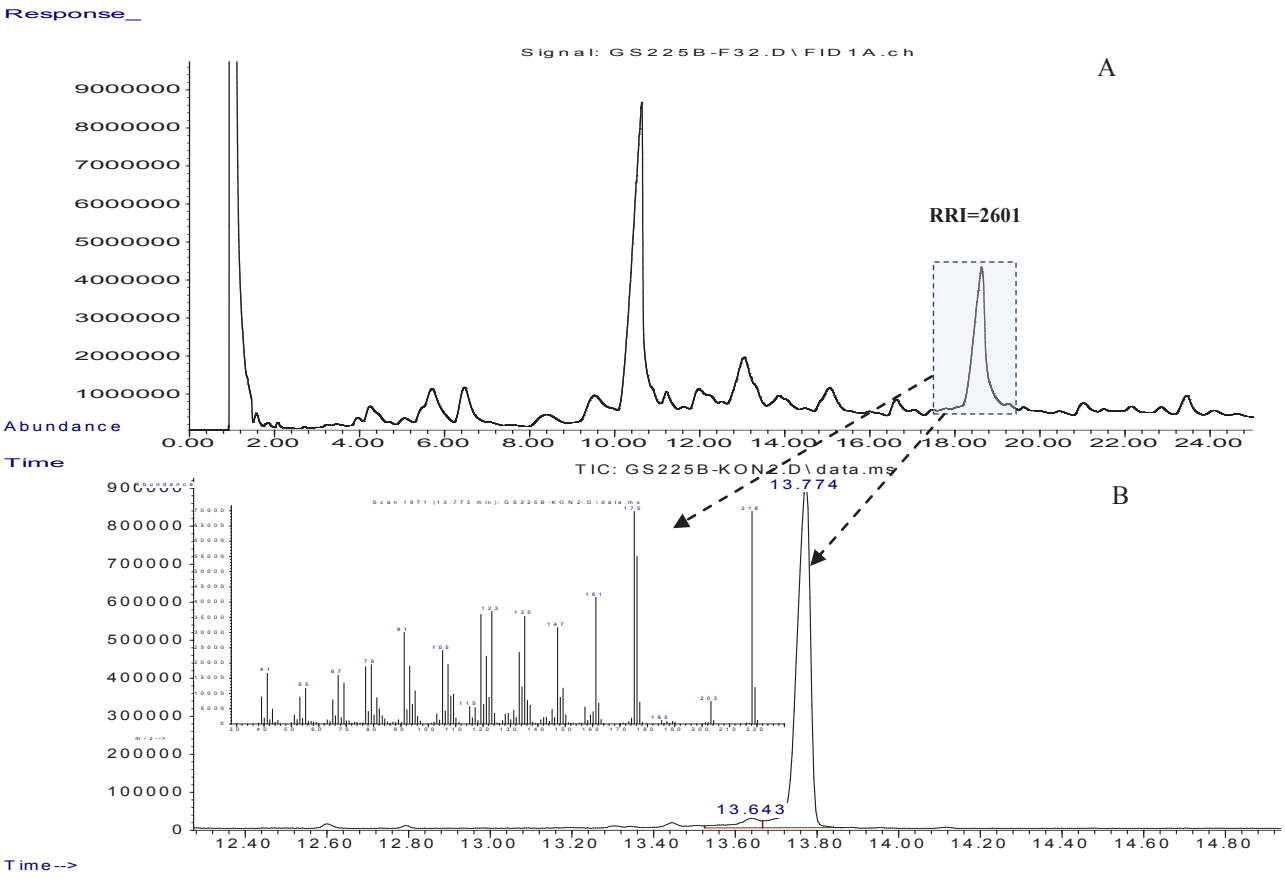

Figure 2: Preparative gas-chromatographic separation with PFC system of the targeted unidentified constituent **1** [M⁺218] from *P. heyniae* EO1. **A**: The shaded area represents peak selected for trapping by PC-GC technique: between 18.40 min and 19.00 min. **B**: Control of the isolated constituent for purity using GC-MS and GC/FID.

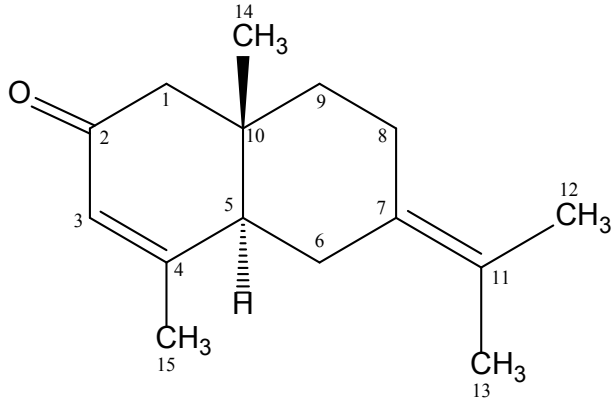

Figure 3: Structure of compound **1** (3,7(11)-eudesmadien-2-one).

unsaturated ketone system in the structure. The proton and carbon data, secured by COSY, HSQC and HMBC spectra, revealed a characteristic eudesmane-type sesquiterpene framework [62]. Inspection of the COSY spectrum revealed three spin systems: i) H-3 to H_3-15 and H-5 (allylic coupling) H-5 to H_2-6 (δ_H 5.86 1.92/2.25 1.71/2.86); ii) H_2-8 to H_2-9 (δ_H 1.94/2.55 1.32/1.52); iii) H_2-1 (2.13 2.24, AB system) (**Figure 2**). Based on the HMBC correlations, positions of the spin systems, quaternary carbons and methyl groups were established (**Figure 4**). Firstly, the α,β-unsaturated ketone system was located at C-2/C-3/C-4 of the ring A based on the key HMBC's from C-2 (δ 198.9) and C-5 to H_2-1, from C-5 (δ 48.3) and C-15 (δ 21.8) to H-3 (δ 5.86), and C-3 (δ 126.9), C-4 (δ 163.0) and C-5 to H_3-15 (δ 1.92). The second olefinic system with tetrasubstitution pattern was located between C-7 and C-11 due to $^3J_{C\text{-}H}$ long-range correlations from C-7 (δ 129.5) to H_3-12 (δ 1.67) and H_3-13 (δ 1.69), and from C-11 (δ 123.0) H_2-8 (δ 1.94 and 2.55) and H_2-6 (δ 1.71 and 2.86). Additionally, the position of the methyl group resonated in the up-field (δ 0.93) was assigned to CH_3-14 on the basis of the long-range correlations from C-1, C-5 and C-9 to H_3-14 (**Table 5**).

The relative stereochemistry of **1** was resolved by the 2D-NOESY spectrum. The cross nOe peaks between H_3-14 (δ 0.93) and H-1_{eq} (δ 2.24), and from the other H-1 proton

Table 5: ^{1}H (600 MHz) and ^{13}C (150 MHz) NMR assignments of **1** in $CDCl_3$.

	δ_C	δ_H (*J* in Hz)
1	54.3	2.13 *d* (16.2); 2.24 *d* (16.2)
2	198.9	
3	126.9	5.86 *s*
4	163.0	
5	48.3	2.25 *m*
6	27.0	2.86 *dd* (13.8, 1.8); 1.71 *m* (13.8)
7	129.5	
8	24.7	2.55 *dt* (1.8, 15.0); 1.94 *m*
9	40.6	1.52 *m*; 1.32 *td* (4.2, 13.2)
10	37.3	
11	123.0	
12	20.2	1.67 *s*
13	20.1	1.69 *s*
14	16.3	0.93 *s*
15	21.8	1.92 *s*

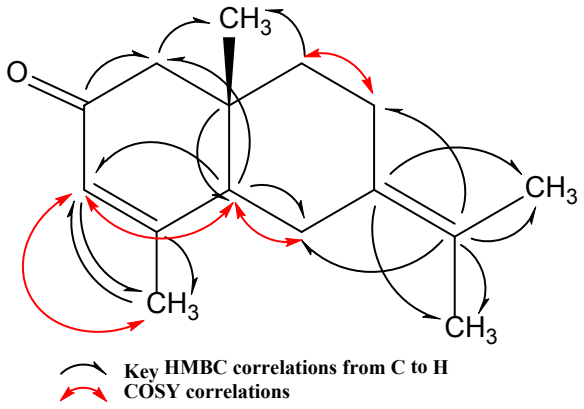

Figure 4: Key HMBC and COSY correlations of compound **1.**

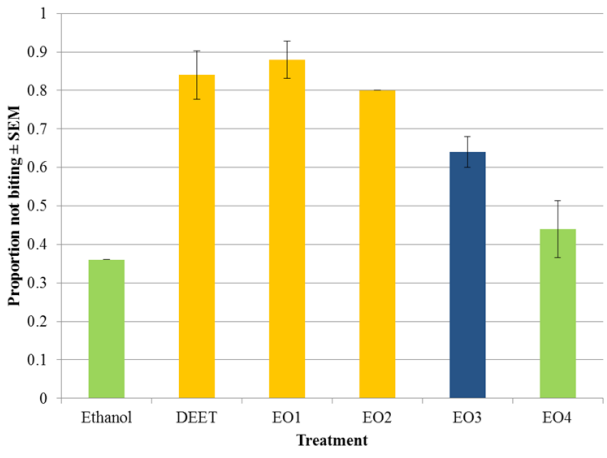

Figure 5: Proportion not biting values of *P. heyniae* essential oil samples at 10 µg/cm² against female *Ae. aegypti*. DEET at 25 nmol/cm², was used as positive control. Ethanol was used as solvent control.

(δ 2.13, axial oriented) to H-5 (δ 2.25) substantiated the trans-diaxial orientation of the H-5 and CH_3-14. Based on the biogenetic grounds, the configuration of C-14 was assumed to be β, designating H-5 as alpha-oriented (Supplementary material). Consequently, the structure of **1** was determined as 3,7(11)-eudesma-dien-2-one ($[\alpha]^{25}_D$: +83º (*c* 0.9, $CHCl_3$), a new natural product. The series of IR absorptions was observed at 3390, 2918, 1667, 1436, 1314, 1014, 952 cm^{-1}. A literature search revealed that eudesmane ketones have earlier been reported for *Citrus paradisi* Macfayden [63]. In a previous study of *P. heyniae* EO [19] has shown that there was no information about eudesmane type sesquiterpene ketone in *Prangos* EO. This is the first report about isolation of an eudesmadienone type constituent from *Prangos* species.

3.2 Mosquito activity

Through the Deployed War-Fighter Protection (DWFP) Research Program, we have expanded our role in exploration and identification of new natural compounds for biting-deterrent and larvicidal activity. Ultimately, our goal is to find new insecticides with low mammalian and environmental toxicity. The *in vitro* biting-deterrence bioassay quantified for the mosquito biting-deterrent properties of *Prangos* EOs against female *Ae. aegypti*. The proportion not biting values of *P. heyniae* EOs were 0.88 (EO2), 0.68 (EO1), 0.64 (EO3) and 0.44 (EO4) (**Figure 5**). PNB values for DEET and ethanol were 0.84 and 0.36.
Data on the toxicity of *P. heyniae* EOs (EO1-4) against first-instar larvae of *Ae. aegypti* at 24-hours post treatment are given in **Figure 6.** Mortality at 125 ppm was 100% in all EO1-4 samples whereas EO1 and EO2 showed 100% mortality at 62.5 ppm, and EO3 and EO4 had 70% and 50% mortality, respectively. At a 31.25 ppm dose, mortality was 30, 90, 40 and 0% for EO1-4 and at the lowest dose (15.625 ppm), none of EOs showed mortality. Permethrin showed 100% mortality at 0.025 ppm and DMSO was 0% for the larvicidal bioassays.

A literature search revealed the repellency and insecticidal effectiveness of several *Prangos* species. In our previous study, we have reported mosquito activity of suberosin isolated from the fruit oil of *P. pabularia* Lindl against *Ae. aegypti*. [40]. The essential oil residues of *P. acaulis* (Dc.) Bornm was active against *Tribolium castaneum* (Herbst), *Sitophilus oryzae* (L.) and *Callosobruchus maculatus* (F.) [64]. The results obtained by Sumer [39] indicated that *P. ferulacea* EO should be used as a control agent against *Ephestia kuehniella* for an integrated pest management program. Several sesquiterpenes showed

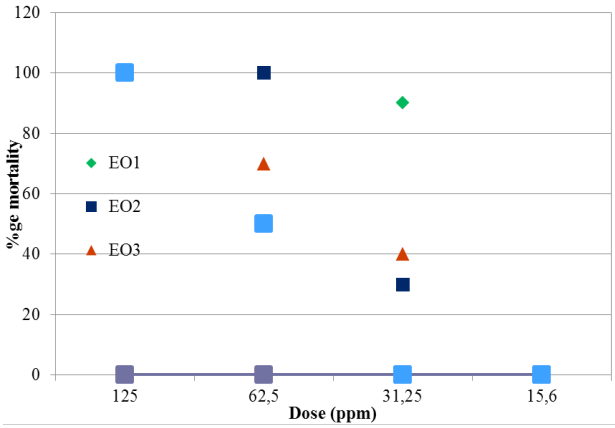

Figure 6: Percent mortality at various dosages of *P. heyniae* EO1-4 against first instar *Ae. aegypti*. DMSO was used as solvent control.

noteworthy insecticidal activities. For example, elemol, an oxygenated sesquiterpene, showed excellent promise as a mosquito repellent with comparable activity to DEET in contact and residual repellency [65-67]. In an another study, β-elemene, sesquiterpene hydrocarbon, showed repellency with a minimum effective dosage (MED) of 0.23 ± 0.14 mg/cm^2 (DEET was 0.008 ± 0.001 mg/cm^2) in human based repellent bioassays against female *Ae. aegypti* [67]. In a recently published study by Pavela claimed that the EOs containing compounds of the sesquiterpene group (α-cadinol, germacrene D, β-caryophyllene) can be recommended as being highly promising for the development of botanical larvicides. It is thus likely that mosquito larvae are more sensitive to sesquiterpenes than monoterpenes [68].

4 Conclusion

In this study, a new eudesmane type sesquiterpene ketone, 3,7(11)-eudesma-dien-2-one (**1**), was identified and was found for the first time for *Prangos* essential oils. Preparative gas chromatography proved a useful tool for isolation of target compound(s) with high purity from the complex mixture like essential oils. This study also compares the chemical composition of four *P. heyniae* EOs and their biting-deterrent and larvicidal activities against *Ae. aegypti*. Based on these results, *P. heyniae* EO1 was the most active essential oil in both assays. Since compound **1** is present only in EO1, its mosquito activity is promising to evaluate in further studies. Recent studies have further proven the effectiveness of terpenes as alternatives for arthropod repellents [69-72]. There is increasing demand in developing of botanical repellents. Research and development of alternative repellent compounds for mosquito control would also serve a valuable role in consumer acceptance and the overall movement towards integrated management of arthropods that transmit diseases.

Disclaimer: All authors of the manuscript declare that they do not have financial/commercial conflicts of interest.

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