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# The Identification of Suberosin from *Prangos pabularia* Essential Oil and Its Mosquito Activity Against *Aedes aegypti*

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**Abstract:** A detailed analysis of *Prangos pabularia* Lindl. (Apiaceae) fruit oil was performed by gas chromatography (GC-FID) and gas chromatography-mass spectrometry (GC-MS). Bicyclogermacrene (21%), (*Z*)-β-ocimene (19%), α-humulene (8%), α-pinene (8%) and spathulenol (6%) were the main constituents of the oil. One compound with 1.8% at RI 3420 remained unidentified or tentatively identified as suberosin from the Wiley GC-MS Library. The assumed compound, suberosin was synthesized in two steps and its structure was confirmed by 1D NMR and GC-MS analyses. As part of our continued research to discover new chemicals for use in mosquito control agents as repellents and larvicides, suberosin and its parent compound coumarin were investigated for the mosquito biting deterrent and larvicidal activity against *Aedes aegypti*. Both suberosin and coumarin showed biting deterrent activity but the activity was lower than the positive control, DEET (*N*,*N*-diethyl-3-methylbenzamide). In larval bioassays, suberosin with $LC_{50}$ value of 8.1 ppm was significantly more toxic than coumarin ($LC_{50}$ = 49.6 ppm) at 24-h post treatment. These results indicate that suberosin may be useful for use as mosquito larvicidal agent.

**Keywords:** *Prangos*; suberosin; coumarin; deterrent activity; larvicidal activity; *Aedes aegypti*. 

## 1. Introduction

The genus *Prangos* Lindl. (Apiaceae) comprises 43 taxa in the worldwide where it is mainly characterized by central and west Asia and Mediterranean regions and the Anatolian flora is an important centre for *Prangos* which has 16 taxa recorded in Turkey [1-3]. The *Prangos* has a long

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historical medical record starting from De Materia Medica by Dioscorides and *P. ferulacea* (L.) Lindl. was reported under the name "Ippomarathon", for the treatment of the kidney and the urinary tract diseases [4]. *Prangos ferulacea* and *P. pabularia* Lindl. are the most widespread species in Turkey; they both are locally known as "Casir" or Caksir", and their decoctions are used as stimulants [1,5]. Roots of *Prangos* species are also used in Turkey as aphrodisiac like *Ferula* and *Ferulago* species [1,3,6]. As external application, *P. platychlaena* Boiss. et Tchihat. has been used to stop bleeding in skin or to heal the scars in eastern Turkey [7]. In Iran, *P. pabularia* called "Djashire-Ulufei" and root extracts have been used as diuretic [8] and *P. ferulacea* is locally known as "Jashir" which is traditionally used as flavoring additive in yogurt in the Mediterranean and Middle-east regions [9-10]. *Prangos pabularia* spread further to central Asia and fruits were used as carminative, stimulant and diuretic in Amhi system which is a traditional healing system in Himalayas [11]. The roots of *P. pabularia* ("Komal") have been used for emmenagogue in India and the leaf of *P. ferulacea* ("Jashir") have been used for abortifacient in Iran to possess antifertility effects [12]. *Prangos peucedanifolia* Fenzl. which is locally known as "Karkol" has been used for treating kidney disorders, bladder inflammation and hemorrhoids in Iraq [13]. An infusion of aerial parts from *P. asperula* Boiss. has been reported to treat reduced blood pressure, skin disease, digestive disorders and hemorrhoids in Lebanon [14]. *Prangos tschimganica* B. Fedtsch. has been used as a folk medicine for the treatment of leukoplakic disease in Uzbekistan [15]. Literature survey revealed that alkaloids, coumarins, flavonoids and terpenoids were isolated from various *Prangos* species [6-7, 15-17]. *Prangos* extracts and some of isolated compounds have been investigated for biological activities including antibacterial [7,16], antispasmodic affects [9-10] anti-inflammatory effects [16], antioxidant [18,20], and cytotoxicity effects [19,20]. Literature survey revaluated that the chemical variation among *Prangos* species showed quiet diversity in the oils can be explained the diverse geographical conditions, distillation techniques and genetic factors [3,6,8,13,21- 40].

As part of our ongoing research project on phytochemical investigation of medicinal and aromatic plants, chemical and medicinal properties of *P. pabularia* prompted to look into a detailed investigation. The fruit oil was analyzed by gas chromatography (GC) and gas chromatography-mass spectrometry (GC-MS) and an unknown compound in the oil was identified by 1D NMR and GC-MS analyses. In addition, this compound was investigated for its biting deterrent and larvicidal activity against *Aedes aegypti* L.

## 2. Materials and Methods

### *2.1. Plant material and essential oil isolation*

*Prangos pabularia* fruits were collected from Van province (Emek village), the eastern part of Turkey. Voucher specimen was deposited at the Herbarium of Selcuk University, Department of Biology Education, Konya Turkey (Voucher number: A. Duran 7519). The air dried fruits were subjected to hydrodistillation for 3 h using a Clevenger-type apparatus [41] to produce an essential oil at a yield of 0.22% as moisture free basis.

### *2.2. Gas chromatography and gas chromatography–mass spectrometry analysis*

The GC-MS analysis was carried out with an Agilent 5975 GC-MSD system (Agilent, USA; SEM Ltd., Istanbul, Turkey). The HP-Innowax FSC column (60 m × 0.25 mm id with 0.25 μm film thickness, Agilent, USA) was used with a helium carrier gas at 0.8 mL/min. The GC oven temperature was kept at 60°C for 10 min and then programmed to 220°C at a rate of 4°C/min and maintained constant at 220°C for 10 min and then programmed to increase at a rate of 1°C/min to 240°C and kept at 240°C for 35 min. Total of analysis took in 115 min. The oil was analyzed with a split ratio of 40:1. The scanned mass spectra were recorded at 70 eV. Mass range was from *m/z* 35 to 450 amu. The GC-FID analysis was carried out with capillary GC using an Agilent 6890N GC system (SEM Ltd., Istanbul, Turkey) with the same column and under same analysis conditions. GC injection port temperature and FID detector temperatures were 250°C and 280°C simultaneously. Interface

temperature was 280°C. Relative percentage amounts of the separated compounds were calculated from the FID chromatograms.

### *2.3. Identification and quantification of compounds*

Compounds were identified by comparison of the chromatographic peaks retention times those of authentic compounds analyzed under the same conditions, and by comparison of 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 3.0 [42], Adams Library [43], 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 analyses of the oils.

### *2.4. Synthesis of suberosin*

Melting points were determined on a hot-stage apparatus and are uncorrected. Nuclear magnetic resonance (NMR) analyses were performed at Nucleic Magnetic Resonance Facility of the University of Florida. NMR spectra were recorded in $CDCl_3$ with TMS as the internal standard for $^1H$ (500 MHz) or $CDCl_3$ as the internal standard for $^{13}C$ (125 MHz). The reactions were carried out under.

Suberosin (**2a**) was synthesized in two steps (Figure 1): On the first step 4-methoxy-2-((3-methylbut-2-en-1-yl)oxy)benzaldehyde (**1**) was prepared by prenylation of 2-hydroxy-4-methoxybenzaldehyde in dimethylformamide (DMF) in the presence of sodium hydride using 3,3-dimethylallyl bromide as described in Coombes et al. [44], then suberosin (**2a**) was prepared by refluxing of mixture of 4-methoxy-2-((3-methylbut-2-en-1-yl)oxy)benzaldehyde (**1**) and ethyl 2-(triphenylphosphoranylidene)acetate in *N*,*N*-dimethylaniline for 8 h [45].

Procedure for 4-Methoxy-2-((3-methylbut-2-en-1-yl)oxy)benzaldehyde synthesis (**1**): Sodium hydride (60% dispersion in mineral oil, 6.15 mmol) was added to a stirred solution of 2-hydroxy-4-methoxybenzaldehyde (5.6 mmol) in DMF (25 mL) under argon at 0 °C [44]. After 5 min of stirring 3,3-dimethylallyl bromide (6.75 mmol) was added. The reaction mixture was then warmed to room temperature and stirred for 1 h. Saturated ammonium chloride solution (100 mL) was added and the mixture was extracted with ether (3 x 100 mL). The combined organic extracts were further washed with water (4 x 100 mL), dried ($Na_2SO4$), and concentrated under reduced pressure to yield the desired compound 4-methoxy-2-((3-methylbut-2-en-1-yl)oxy)benzaldehyde (**1**), that was used without further purification.

4-Methoxy-2-((3-methylbut-2-en-1-yl)oxy)benzaldehyde (1): Off white crystals [45], mp 40-41 ºC; yield 99%; $^1H$ NMR ($CDCl_3$) $\delta$ 10.31 (s, 1H), 7.80 (d, $J$ = 8.7 Hz, 1H), 6.58-6.50 (m, 1H), 6.46-6.43 (m, 1H), 5.53-5.46 (m, 1H), 4.60 (d, $J$ = 6.4 Hz, 2H), 3.85 (s, 3H), 1.80 (s, 3H), 1.75 (s, 3H). $^{13}C$ NMR ($CDCl_3$) $\delta$ 188.4, 166.0, 163.1, 138.7, 130.3, 118.9, 118.8, 105.8, 98.9, 65.4, 55.5, 25.7, 18.2.

Procedure for suberosin (**2a**) synthesis: A mixture of 4-methoxy-2-((3-methylbut-2-en-1-yl)oxy)benzaldehyde (**1**) (1.5 mmol) and ethyl 2-(triphenylphosphoranylidene)acetate (1.7 mmol) in *N*,*N*-dimethylaniline (25 mL) was refluxed, under nitrogen atmosphere, for 8 h. Excess of aniline was removed under reduced pressure [45]. The residue obtained was extracted with ethyl acetate (3 × 10 mL). The combined extract was washed with diluted HCl and water, dried over anhydrous $Na_2SO_4$, and concentrated. The silica gel column chromatography using hexanes and ethyl acetate as gradient eluent gave the desired product suberosin (**2a**). In earlier fractions 3-prenyl-7-methoxycoumarin (**2b**) was obtained in minor amount.

Suberosin (**2a**): Yellowish crystals [45] after chromatography on silica gel using hexanes/ethyl acetate as eluent (100/0–2, v/v); mp 88-89 ºC; yield 48%; $^1H$ NMR ($CDCl_3$) $\delta$ 7.60 (d, $J$ = 9.4 Hz, 1H), 7.16 (s, 1H), 6.74 (s, 1H), 6.21 (d, $J$ = 9.5 Hz, 1H), 5.31-5.23 (m, 1H), 3.87 (s, 3H), 3.29 ( d, $J$ = 7.4 Hz, 2H), 1.75 (s, 3H), 1.69 (s, 3H). $^{13}C$ NMR ($CDCl_3$) $\delta$ 161.5, 160.6, 154.4, 143.6, 133.6, 127.4, 127.3,

121.3, 112.6, 111.8, 98.4, 55.8, 27.7, 25.7, 17.7. EIMS, *m/z:* 244 [$M^+$] $C_{15}H_{16}O_3$ (67.4), 39 (1.5), 55 (1.5), 89 (3.5), 103 (3.7), 115 (7.0), 128 (6.2), 141 (4.4), 159 (7.3), 175 (6.2), 189 (14.1), 201(6.0), 213 (5.0), 229 (100.0), 230 (15.8).

3-Prenyl-7-methoxycoumarin as off white crystals (**2b**): Off white crystals [45] after chromatography on silica gel using hexanes/ethyl acetate as eluent (100/0–2, v/v); mp 91-92 ºC; yield 7%; $^1$H NMR ($CDCl_3$) $\delta$ 7.38 (s, 1H), 7.32 (d, $J$ = 8.4 Hz, 1H), 6.89-6.79 (m, 2H), 5.38-5.23 (m, 1H), 3.85 (s, 3H), 3.22 (d, $J$ = 6.8 Hz, 2H), 1.80 (s, 3H), 1.69 (s, 3H). $^{13}$C NMR ($CDCl_3$) $\delta$ 162.2, 161.7, 154.6, 138.1, 135.4, 128.0, 125.3, 119.4, 113.3, 112.3, 100.4, 55.7, 28.6, 25.8, 17.8. EIMS, *m/z:* 244 [$M^+$] $C_{15}H_{16}O_3$ (52.1), 41 (3.4), 77 (4.1), 115 (6.5), 118 (4.7), 137 (5.2), 161 (8.3), 189 (100.0), 190 (11.5), 201 (22.1), 215 (5.0), 229 (25.1).

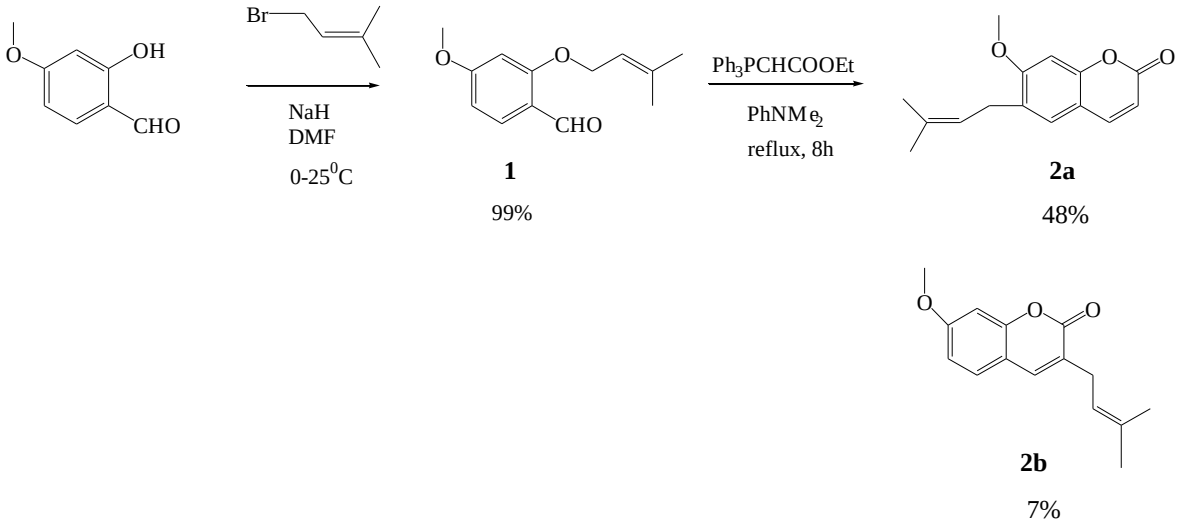



**Figure 1**. Synthesis of suberosin (**2a**)

## *2.5. Insects*

*Aedes aegypti* (L.) used in these studies were from a laboratory colony maintained since 1952 at the Mosquito and Fly Research Unit at Center for Medical, Agricultural and Veterinary Entomology, USDA-ARS, Gainesville, Florida. This colony is maintained using standard procedures.

### *2.5.1. Mosquito Biting Bioassays*

Mosquitoes were reared to the adult stage by feeding larvae a diet of 2% slurry of 3:2 beef liver powder and Brewer's yeast. Eggs on a piece of paper were hatched in a cup filled with 100 mL of de-ionized water containing a small quantity of larval diet. These larvae were then transferred into 500 mL cups (about 100 larvae per cup) filled with water. Larval diet was added every day until pupation, and the mosquitoes were kept in an environmentally-controlled room at a temperature of 27 ± 2˚C and 60 ± 10 % RH with a photoperiod of 12:12 (L: D) h. Adult mosquitoes were fed from cotton pads moistened with 10% sucrose solution placed on the top of screens of 4 L cages. Eight to 15-day-old mated females used in these bioassays were deprived of sucrose for 24 h prior to the test; but had free access to water-soaked cotton. A six-celled Klun & Debboun (K & D) module bioassay system [46] was used to quantify the biting deterrence of pure compounds, suberosin (**2a**) and coumarin (CAS # 91-64-5) which was purchased from Sigma-Aldrich Co., St. Louis, MO, USA.

The term deterrent refers to a chemical that inhibits feeding when present in a place where the insects feed in its absence and the repellent is a chemical that causes insects to make oriented movement away from its source [47]. The K & D system consists of a six-well reservoir with each of

the 4 x 3 cm wells containing 6 mL of feeding solution. As reported previously by Ali et al. [48], we used the citrate-phosphate-dextrose-adenine (CPDA-1) + ATP solution instead of human blood. CPDA-1 and ATP preparations were freshly made on the day of the test and the mixture contained a red dye for verifying whether mosquitoes had imbibed the solution. DEET (97% purity *N*,*N*-diethyl-*meta*-toluamide) was obtained from Sigma-Aldrich and used as a positive control. Molecular biology grade ethanol was obtained from Fisher Scientific Chemical Co. (Fairlawn, NJ 07410). Stocks and dilutions of essential oils, sub-fractions, individual compounds and DEET were prepared in ethanol. Stock solutions were kept in a refrigerator set at 3-4°C. During the bioassay, temperature of the solution in the reservoirs covered with a collagen membrane was maintained at 37.5°C by circulating water through the reservoir with a temperature-controlled circulatory bath. The test compounds and controls were randomly applied to six 4 x 3 cm marked portions of nylon organdy strip, which was positioned over the six, membrane-covered wells. A teflon separator was placed between the treated cloth and the module. The K & D system with six-well reservoir containing five females of *Ae. aegypti* per cell was positioned over the wells. Trap doors were opened and mosquitoes allowed access for a 3-minute period, after which they were collected back into the module. Mosquitoes were squashed and the presence of red dye in the gut was used as an indicator of feeding. A replicate consisted of six treatments: four treatments (samples), DEET (positive control) and 100% ethanol (solvent control). Five replicates were conducted per day using new batches of mosquitoes for each. Bioassays were conducted between 13:00 and 16:00 h and 10 replications were conducted for each treatment.

### *2.5.2. Mosquito Larvicidal Bioassays*

Eggs were received and stored in a laboratory (Biological Field Station, The University of Mississippi, Abbeville, MS) until needed. Bioassays were conducted using the system described by Pridgeon et al. [49] to determine the larvicidal activity of individual compounds and essential oils against *Ae. aegypti*. Eggs were hatched and larvae were held in a room maintained at a temperature of 27 ± 2°C with 60 ± 10% RH. Five 1-day-old larvae were transferred to individual wells of a 24-well tissue culture plates in a 30-40 µL droplet of water. Fifty µL of larval diet of 2% slurry of 3:2 beef liver powder (Now Foods, Bloomingdale, Illinois) and Brewer's yeast (Lewis Laboratories Ltd., Westport, CT) and 1 mL of deionized water were added to each well by using a Finnpipette stepper (Thermo Fisher, Vantaa, Finland). Suberosin (**2a**) and coumarin were diluted in DMSO. Eleven microliters of the test chemical was added to the labeled wells, while 11 µL of DMSO was added to control treatments. After 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 tested compounds. Larval mortality was recorded 24 and 48 h post treatment. Larvae were deemed dead if they showed no movement in the well after being prodded with a pipette tip. A series of 5 dosages were used in each treatment to get a range of mortality between 0 to 100%. Treatments were replicated 10-20 times for each samples. Permethrin (Chemical Service, West Chester, PA) at 0.025 ppm was used as positive control.

### *2.5.3. Statistical Analysis*

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

$$\text{PNB} = 1 - \left(\frac{\text{Total number of females biting}}{\text{Total number of females}}\right)$$

Proportion not biting data were analyzed using SAS Proc ANOVA [50] and means were separated using the Ryan-Einot-Gabriel-Welsch Multiple Range Test. Control mortality was corrected by using Abbott's formula. $LD_{50}$ values for larvicidal data were also calculated by using SAS, Proc Probit.

## 3. Results and Discussion

### *3.1. Determination of essential oil components*

Water distilled essential oil from *P. pabularia* fruits was analyzed by GC-FID and GC-MS systems. Identified 60 components made up 93.2% of the total oil (Table 1). Sesquiterpenes were the most dominant class of compounds in the oil with bicyclogermacrene (21%), α-humulene (8%), spathulenol (6%) and α-bisabolol (4%). Monoterpenes [(*Z*)-β-ocimene (19%) and α-pinene (8%)] were the next abundant class in the fruit oil.

**Table 1.** The composition of *Prangos pabularia* fruit essential oil.

| RRI | Compound | % | Methods of identification |
|---|---|---|---|
| 1032 | α-Pinene | 8.0 | MS, RI, std |
| 1035 | α-Thujene | < 0.1 | MS, RI, std |
| 1048 | 2-Methyl-3-buten-2-ol | 0.1 | MS, std |
| 1076 | Camphene | 0.1 | MS, RI, std |
| 1093 | Hexanal | 0.1 | MS, RI, std |
| 1118 | β-Pinene | 0.5 | MS, RI, std |
| 1132 | Sabinene | 0.1 | MS, *RI*, std |
| 1137 | Thuja-2,4(10)-diene | < 0.1 | MS, RI, std |
| 1159 | δ-3-Carene | 0.1 | MS, RI, std |
| 1174 | Myrcene | 0.4 | MS, RI, std |
| 1176 | α-Phellandrene | 0.7 | MS, RI, std |
| 1203 | Limonene | 0.6 | MS, RI, std |
| 1218 | β-Phellandrene | 0.7 | MS, RI, std |
| 1246 | (*Z*)-β-Ocimene | 18.8 | MS, RI, std |
| 1266 | (*E*)-β-Ocimene | 2.5 | MS, RI, std |
| 1280 | *p*-Cymene | 0.5 | MS, RI, std |
| 1290 | Terpinolene | 0.4 | MS, RI, std |
| 1382 | *cis*-Alloocimene | 0.1 | MS, RI, std |
| 1384 | α-Pinene oxide | 0.2 | MS, RI, std |
| 1413 | Rosefuran | 0.1 | MS, RI, std |
| 1452 | α,*p*-Dimethylstyrene | 0.1 | MS, RI, std |
| 1455 | 1-Methoxy-2-methyl benzene | 0.3 | MS, RI, std |
| 1483 | (Z)-β-Ocimene epoxide | 0.1 | MS, RI, std |
| 1492 | Bicycloelemene | 0.2 | MS, RI, std |
| 1504 | Daucene | 0.7 | MS, RI, std |
| 1528 | α-Bourbonene | 0.1 | MS, RI, std |
| 1535 | β-Bourbonene | 0.6 | MS, RI, std |
| 1562 | Isopinocamphone | < 0.1 | MS, RI, std |
| 1589 | β-Ylangene | 0.1 | MS, RI, std |
| 1590 | Bornyl acetate | 0.2 | MS, RI, std |
| 1595 | Isothymol methyl ether | < 0.1 | MS, RI, std |
| 1600 | β-Elemene | 0.2 | MS, RI, std |
| 1612 | β-Caryophyllene | 0.8 | MS, RI, std |
| 1615 | 4,4-Dimethylbut-2-enolide | *< 0.1* | MS, RI, std |
| 1628 | Aromadendrene | 0.1 | MS, RI, std |
| 1661 | α-Himachalene | 0.1 | MS, RI, std |
| 1668 | (*Z*)-β-Farnesene | 0.2 | MS, RI, std |
| 1687 | α-Humulene | 8.3 | MS, RI, std |

| RRI | Compound | % | Methods of identification |
|---|---|---|---|
| 1704 | γ-Muurolene | 0.5 | MS, RI, std |
| 1708 | Ledene | 0.2 | MS, std |
| 1726 | Germacrene D | 2.6 | MS, RI, std |
| 1727 | 7-*epi*-1,2-Dehydrosesquicineole | 3.2 | MS, RI, std |
| 1730 | Dehydro-α-Curcumene | 0.5 | MS, RI, std |
| 1740 | β-Bisabolene | 0.2 | MS, std |
| 1755 | Bicyclogermacrene | 20.9 | MS, RI, std |
| 1773 | δ-Cadinene | 0.4 | MS, RI, std |
| 1783 | β-Sesquiphellandrene | 0.1 | MS, RI, std |
| 1784 | (*E*)-α-Bisabolene | 0.6 | MS, RI, std |
| 1787 | Aromadendra-1(10),4(15)-diene | 0.1 | MS, RI, std |
| 1815 | 2,6-Dimethyl-3(*E*), 5(*Z*), 7-octatriene-2-ol | 0.2 | MS, RI, std |
| 1830 | 2,6-Dimethyl-3(*E*), 5(*E*), 7-octatrien-2-ol | 0.3 | MS, RI, std |
| 1942 | 4-Hydroxy-2-methyl acetophenone | *0.9* | MS, RI, std |
| 2008 | Caryophyllene oxide | 0.2 | MS, RI, std |
| 2045 | Humulene epoxide-I | < 0.1 | MS, RI, std |
| 2071 | Humulene epoxide-II | 0.6 | MS, RI, std |
| 2081 | Humulene epoxide-III | 0.2 | MS, RI, std |
| 2094 | *p*-Cresol | 0.6 | MS, RI, std |
| 2098 | Globulol | 0.1 | MS, RI, std |
| 2104 | Viridiflorol | 0.2 | MS, RI, std |
| 2144 | Spathulenol | 6.4 | MS, RI, std |
| 2232 | α-Bisabolol | 4.3 | MS, RI, std |
| 2253 | *trans*-α–Bergamotol | 0.8 | MS, std |
| 2316 | Decanoic acid | 0.2 | MS, std |
| 2931 | Hexadecanoic acid | 1.0 | MS, RI, std |
| 3420 | Suberosin* | 1.8 | MS, NMR, std |
| | **Total** | **93.2** | |

RRI Relative retention indices calculated against *n*-alkanes; % calculated from FID data; MS identification by comparison of the mass spectrum with those of the computer mass libraries (Wiley, NIST, MassFinder-4, Adams, and Baser libraries); RI identification by comparison of Retention Index with those reported in literature; std identification by comparison of the retention time and mass spectrum of authentic standard; * suberosin was synthesized in two steps and its structure was confirmed by 1D NMR and GC-MS analyses.

The identification of the separated volatile compounds was accomplished through comparison of mass spectra of peaks and retention indices (RI) of standards stored in the in-house library (Başer Library of Essential Oil Constituents). From the GC-MS analysis, the peak at RI 3420 was not identified with 'Başer Library of Essential Oil Constituents', however, its mass spectrum was similar to suberosin according to the Wiley GC-MS Library. Since this compound was not present in our standard essential oil library, we could not prove the identity of the compound, based only on similarity to the one matching suberosin mass spectrum in Wiley GC-MS Library; also, due to the insufficient oil yield, we were not able to isolate the assumed compound; therefore, we synthesized the suberosin. Synthesis was carried out in two steps (Figure 1). The prenylation of 2-hydroxy-4-methoxybenzaldehyde in dimethylformamide with 3,3-dimethylallyl bromide at 0ºC in the presence of sodium hydride [44] gave 4-methoxy-2-((3-methylbut-2-en-1-yl)oxy)benzaldehyde (**1)** in 99% yield. Then **1** was refluxed with ethyl 2-(triphenylphosphoranylidene)acetate in *N*,*N*-dimethylaniline [45] to give the desired product suberosin (**2a**), which was isolated from the reaction mixture by silica gel column chromatography (48% yield). As a co-product, 3-prenyl-7-methoxycoumarin (**2b**) was isolated in 7% yield. The identification of suberosin was performed by 1D NMR; the interpretations of proton and carbon signals were consistent with the literature data [45]. The synthesized compounds **2a** and **2b** were subjected to GC-MS analysis under the same GC conditions as was used for *P. pabularia* oil. The peak at the 111 minute was match with suberosin (**2a**) (Figure 2). Compounds **2a** and **2b were**

added to the ‘’Başer Library of Essential Oil Constituents’’ library with RRI 3420 and 3310, respectively.

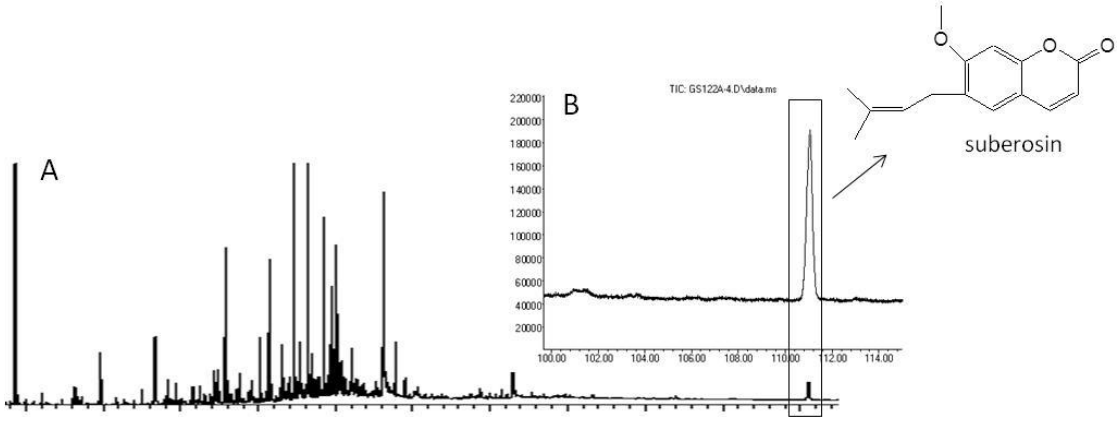



**Figure 2.** Gas chromatography of *P. pabularia* fruit oil (A) and the control of synthesized compound, suberosin (B)

### *3.2. Mosquito deterrent and larvicidal activity*

Mosquitoes are vectors for many diseases, therefore, repellents and insecticides are important for the mosquito control. Through the Department of Defense (DOD) Deployed War-Fighter Protection (DWFP) Research Program, we explore the plant extracts and pure compounds for mosquito repellent and larvicidal activity to find new mosquito control agents. Suberosin is a simple coumarin with prenyl group on the $C_6$ and methoxy group at the $C_7$ on the benzene ring and its parent compound coumarin which is consisting of benzene and α-pyrone ring were investigated for the mosquito biting deterrent and larvicidal activity against *Ae. aegypti*. Both suberosin and coumarin showed biting deterrent activity above the solvent control, and their activity was similar to each other. However, biting deterrent activity for both of them was significantly lower than that for positive control DEET (*N*,*N*-diethyl-3-methylbenzamide) (Figure 3).

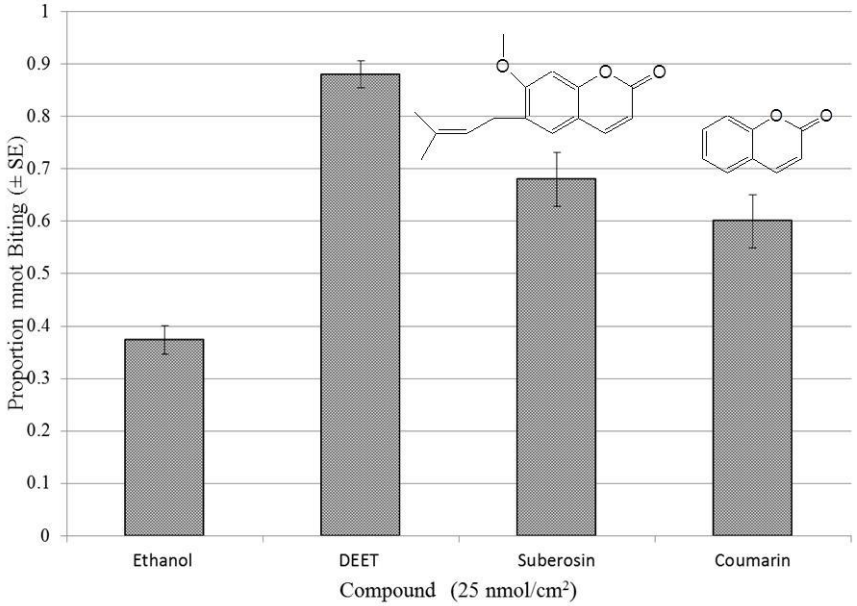



**Figure 3**. Biting deterrent effects of DEET, suberosin and coumarin at 25 nmol/cm$^2$ against *Aedes aegypti*

The chemical composition of various *Prangos* essential oils has been investigated from diverse locations, different plant tissues and different extraction techniques that influenced their essential oil profile (Table 2). Our *P. pabularia* fruit oil results were in agreement with previous study of *P. pabularia* fruit oil [3]. *Prangos pabularia* fruit oil grown in Iran contained mainly α-pinene 33.87%, spathulenol 9.32% and α-santalone 7.05% [8], whereas, in our sample, these compounds were found in less amounts: 4.0%, 6.4% and 0%, respectively. This difference can be expected due to difference in locations where samples were collected.

**Table 2.** The main compounds, distillation techniques and country of origins of different *Prangos* essential oils.

| ***Prangos* Species** | **Distillation Technique** | **Plant part used** | **Main compounds** | **Country** | **References** |
|---|---|---|---|---|---|
| *P. acaulis* | HD | aerial parts | α-pinene 12.5%, *cis*-sabine hydrate 27.1%, γ-eudesmol 7.4%, spathulenol 7.2% | Iran | 21 |
| *P. acaulis* | HD | stems | 3-ethylidene-2-methyl-1-hexen-4-yne 56.8%, α-pinene 34.2% | Iran | 22 |
| | | leaves | α-pinene 39.54%, 3-ethylidene-2-methyl-1-hexen-4-yne 37.94%, α-terpinolene 10.9% | | |
| | | flowers | α-pinene 25.04%, 3-ethylidene-2-methyl-1-hexen-4-yne 23.51%, α-terpinolene 17.26%, limonene 13.64% | | |
| *P. acaulis* | HD | aerial parts | α-pinene 22.87%, limonene 21.36%, 3-ethylidene-2-methyl-1-hexen-4-yne 21.36% | Iran | 23 |
| | SFE | | 3-ethylidene-2-methyl-1-hexen-4-yne 14.3%, α-pinene 13.7%, octadecane 9.4%, eicosane 8.9%, nonadecane 7.3%, limonene 6.1% | | |
| *P. asperula* | HD | fruits | δ-3-carene 16.1%, α-pinene 10.5%, β-phellandrene 14.7, α-humulene 7.8 | Iran | 24 |
| *P. asperula* | HD | aerial parts | δ-3-carene 25.54%, α-terpinolene 14.76%, α-pinene 13.6%, limonene 12.94%, mrycene 8.1%, β-pinene 5.4% | Iran | 25 |
| *P. asperula* | HD | aerial parts | 2,3,6-trimethyl benzaldehyde 18.4%, δ-3-carene 18.0%, α-pinene 17.4% | Iran | 26 |
| *P. bornmuelleri* | LK | fruits | germacrene-4-ol 42.8%, α-cadinol 18.5%, γ-cadinene 10.9% | Turkey | 27 |
| *P. corymbosa* | HD | aerial parts | β-elemene 22.0%, spathuleneol 12.5% | Iran | 28 |
| | | flowers | β-elemene 40.7%, kessane 10.7% | | |
| *P. denticulata* | HD | fruits | sabinene 26.1%, *p*-cymene 19.7% | Turkey | 29 |
| | | roots | δ-3-carene 49.3%, (*Z*)-3,5-nonadiyne,7-ene 20.4% | | |
| *P. ferulacea* | HD | crushed fruits | γ-terpinene 30.22%, α-pinene 16.70%, *p*-cymene 9.85% | Turkey | 30 |
| | | whole fruits | γ-terpinene 33.22%, α-pinene 12.83%, *p*-cymene 10.78% | | |
| | | redistilled whol | germacrene B 30.30%, γ-terpinene 17.17%, germacrene D 7.77% | | |
| | | leaves | linalool 36.7%, caryophyllene oxide 16.3%, α-pinene 12.1% | | |

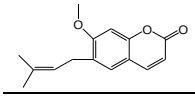
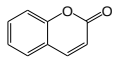
| ***Prangos* Species** | **Distillation Technique** | **Plant part used** | **Main compounds** | **Country** | **References** |
|---|---|---|---|---|---|
| *P. ferulacea* | HD | stem | 1,8-cineole 19.0%, α-pinene 10.3% | Iran | 31 |
| | | flower | linalool 19.0%, lavandullyl acetate 16.0%, 1,8-cineole 14.5%, α-pinene 12.4%, geranyl isobutyrate 12.2% | | |
| *P. heyniae* | HD | fruits location 1 | β-bisabolenal 53.3%, β-bisabolenol 14.6%, β-bisabolene 12.1% | Turkey | 32 |
| | | fruits location 2 | β-bisabolenal 18.0%, germacrene D 13.5%, β-bisabolene 10.1%, germacrene B 9.4% | | |
| *P. latiloba* | HD | leaves | germacrene D 27.79%, α-pinene 17.81%, β-caryophyllene 12.75%, α-pinene 11.23% | Iran | 33 |
| | | stems | γ-cadinene 30.39%, α-pinene 25.47, sabinene 12.55% | | |
| | | roots | spathuleneol 29.5%, 1,8-cineole 19.42%, *p*-cymene 17.03%, α-bisabolol 15.33% | | |
| *P. latiloba* | HS-SPME | flowers | limonene 18.3%, myrcene 10.4%, (*E*)-β-ocimene 7.8% | Iran | 34 |
| | | leaves | limonene 17.5%, β-caryophyllene 12.0%, myrcene 9.4% | | |
| | | stems | limonene 13.5%, myrcene 8.6%, α-phellandrene 4.9% | | |
| *P. pabularia* | HD | fruits | α-humulene 16.6%, bicyclogermacrene 16.1%, spathuleneol 10.6%, germacrene D 5.7% | Turkey | 3 |
| | MD | | α-pinene 23.96%, α-humulene 15.46%, bicyclogermacrene 7.93%, spathulenol 5.70%, germacrene D 2.93% | | |
| *P. pabularia* | HD | leaves | spathulenol 16.0%, α-bisabolol 14.3%, (*Z*)-methoxycinnamaldehyde 9.47% | Iran | 8 |
| | | fruits | α-pinene 33.87%, spathulenol 9.32%, α-santalone 7.05% | | |
| | | umbels | α-pinene 21.46, α-santalene 6.63%, *p*-methoxyacetophenone 5.39% | | |
| *P. peucedanifolia* | HD | leaves | *m*-cresol 50.38%, α-epibisabolol 4.92%, β-pinene 3.63 | Iraq | 13 |
| | | flowers | β-pinene 35.58%, α-pinene 22.13%, β-phellandrene 12.54%, myrcene 8.27%, γ-terpinene 5.97% | | |
| *P. platychlaena* | HD | fruits | α-pinene 69.75%, β-phellandrene 10.58% | Turkey | 35 |
| *P. uechrotzii* | HD | fruits | 7-epi-1,2-dehydrosesquicineole 13.44%, α-pinene 7.85%, β-phellandrene 6.77% | Turkey | 6 |
| | MD | | α-pinene 11.23%, α-phellandrene 8.42%, β-phellandrene 8.26%, *p*-cymene 5.48%, 7-epi-1,2-dehydrosesquicineole 4.56% | | |
| *P. uechrotzii* | HD | fruits | α-pinene 40.82%, β-phellandrene 11.14%, δ-3-carene 7.39% | Turkey | 35 |
| *P. uloptera* | HD | aerial parts | β-caryophyllene 27.1%, caryophyllene oxide 15.9%, α-pinene 12.4% | Iran | 36 |
| *P. uloptera* | HD | umbells | α-pinene 31.78%, β-bourbonene 15.9%, α-curcumene 10.65%, spathuleneol 9.0%, *m*-cymene 5.51% | Iran | 37 |

| ***Prangos* Species** | **Distillation Technique** | **Plant part used** | **Main compounds** | **Country** | **References** |
|---|---|---|---|---|---|
| | | fruits | α-pinene 14.98%, β-bourbonene 7.81%, α-humulene 7.74%, germacrene B 7.23, *n*-tetracosane 6.65% | | |
| *P. uloptera* | HD | aerial parts | δ-3-carene 26.3%, α-pinene 15.4%, myrcene 9.1%, *p*-cymene 8.6%, β-phellandrene 7.6% | Iran | 38 |
| | MA-HS-SDME | | δ-3-carene 32.1%, α-pinene 16.8%, myrcene 11.7%, *p*-cymene 8.5%, β-phellandrene 8.2% | | |
| *P. scabra* | HD | fruits | β-elemene 23.3%, (*Z*)-β-farnesene 16.2%, γ-cadinene 9.96%, β-caryophyllene 9.2%, germacrene B 5.19%, spathuleneol 5.0% | Iran | 39 |
| | | inflorescence | β-elemene 19.7%, epi-globulol 11.5%, caryophyllene oxide 8.98%, α-cadinol 6.2% | | |
| *P. turcica* | HD | fruits | α-humulene 11.0%, germacrene D 10.6%, naphthalene 8.5%, terpinolene 7.9%, bornyl acetate 6.9% | Turkey | 40 |
| | MD | | germacrene D 9.2%, naphthalene 8.7%, bornyl acetate 8.2%, α-humulene 7.1%, β-elemene 6.7% | | |
| | MSD-SPME | | *p*-cymene 12.7%, terpinolene 11.2%, α-pinene 9.9%, naphthalene 7.9%, α-humulene 7.9%, germacrene D 6.2% | | |

HD: Hydrodistillation SFE: Supercritical Fluid extraction MA-HS-SPME: Microwave-assisted headspace single drop micro extraction
MD: Microdistillation HS-SPME: Head space-solid phase-micro extraction MSD-SPME: Micro-steam distilled solid-phase micro extraction
LN: Likens-Nickerson

In larval bioassays, suberosin with $LC_{50}$ and $LC_{90}$ values of 8.1 and 14.0 ppm was significantly more toxic than coumarin having $LC_{50}$ and $LC_{90}$ values of 49.6 and 82.2 ppm, respectively, at 24-h post treatment. Based on 95% CL, toxicity significantly increased from 24 to 48 h post treatment in coumarin whereas level of toxicity did not change in suberosin (Table 3). Suberosin demonstrated better larvicidal than deterrent activity. Coumarins have not been explored much for their insecticidal activity in the literature; however, a few studies on their insecticidal activity were recently reported. Wang et al. studied the structure-activity relationship of six simple coumarins and seven furanocoumarins against *Culex pipiens pallens* and *Ae. aegypti* larvae [51]. They found that furanocoumarins were more toxic than simple coumarins. The furanocoumarins having $C_8$ alkoxyl side chains such as imperatorin and isopimpinellin showed higher toxicity than furanocoumarins lacking $C_8$ alkoxyl side chains. The methoxy group at the $C_5$ or $C_8$ on the furanocoumarins decreased the toxicity level. They also reported that simple coumarin osthole having a $C_8$ prenyl side chain and methoxy group in the $C_7$ showed highest toxicity than other simple coumarins such as coumarin and umbelliferone. An alkoxy substitution in the $C_7$ position on the coumarins such as 7-ethoxycoumarin, herniarin and limettin enhanced the toxicity, whereas hydroxyl group at the $C_7$ on the umbelliferone or simple coumarin nucleus reduced the toxicity [51].

**Table 3.** Toxicity of suberosin and coumarin against *Ae. aegypti* larvae.

| **Compound** | **Time[a]** | **$LC_{50}$ ppm** | **(95%CI)[b])** | **$LC_{90}$ ppm** | **(95% CI)[b])** | **$\chi^2$** | **DF[c])** |
|---|---|---|---|---|---|---|---|
| Suberosin | 24h | 8.1 | (7.4 – 8.9) | 13.99 | (12.4 – | 95.8 | 58 |
|  | 48h | 7.8 | (7.1 – 8.5) | 13.4 | (11.9 – | 98.3 | 58 |
| Coumarin | 24h | 49.6 | (44.5 – | 82.2 | (71.0 – | 60.4 | 38 |
|  | 48h | 27.2 | (24.6 – | 41.8 | (36.6 – | 53.1 | 38 |

[a] Hours post treatment.

[b] $LC_{50}$ and $LC_{90}$ values are given in ppm (95% confidence interval).

[c] DF refers to degree of freedom.

## 4. Conclusions

In this study, *P. pabularia* fruit essential oil composition has been investigated in detail. Tentatively identified suberosin was synthesized and its structure was confirmed by 1D NMR and GC-MS analyses. Natural compounds are source of new classes of insecticides as well as environmentally and toxicologically safer molecules than currently used insecticides. In this respect, suberosin and its parent compound coumarin were investigated *in vitro* deterrent activity against adult female *Ae.aegypti* and larvicidal activity against 1-day old *Ae.aegypti*. To the best of our knowledge, this is the first report on the suberosin found in *P. pabularia* fruit essential oil and tested against *Ae. aegypti* for deterrent and larvicidal activity. We found in this current study that suberosin more deterrent than coumarin and suberosin also showed six times higher toxicity than coumarin against *Ae. aegypti* larvae. Coumarins are an important class of heterocyclic compounds with low molecular weight, and great biological potential, which could vary depending on properties of the functional groups. In case of suberosin, prenyl group at the $C_6$ or methoxy group at the $C_7$ on the benzene ring should be responsible for larvicidal activity against *Ae. aegypti* larvae. Thus, this molecule can be considered as a potential larvicidal agent for further research and development against insect pest.

## Conflict of interest

The authors report no conflicts of interest.

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## Supporting Information

Supporting Information accompanies this paper on http://www.acgpubs.org/RNP

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