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Bioactivity-Guided Investigation of Geranium Essential Oils as Natural Tick Repellents

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Supporting Information

ABSTRACT: The evaluation of 10 essential oils of geranium, *Pelargonium graveolens* (Geraniaceae), were all shown to have repellent activity against nymphs of the medically important lone star tick, *Amblyomma americanum* (L.). The biological tests were carried out using a vertical filter paper bioassay, where ticks must cross an area of the paper treated with repellent to approach host stimuli. One of the essential oil samples that repelled >90% of the ticks at 0.103 mg/cm^2 was selected for further fractionation studies. The sesquiterpene alcohol, (−)-10-epi-γ-eudesmol, was isolated and identified by spectral methods. (−)-10-epi-γ-Eudesmol at 0.103 and 0.052 mg of compound/cm^2 of filter paper repelled 90 and 73.3% of the ticks, respectively. (−)-10-epi-γ-Eudesmol exhibited similar repellency to the reference standard *N*,*N*-diethyl-meta-toluamide (DEET) at concentrations of ≥0.052 mg of compound/cm^2 of filter paper, with (−)-10-epi-γ-eudesmol losing much of its repellency at 0.026 mg of compound/cm^2 and DEET at 0.013 mg of compound/cm^2. Isomenthone and linalool did not repel ticks at the concentrations tested. Most repellents are marketed with much higher concentrations of active ingredient than the concentrations of the natural repellents tested herein; therefore, effective compounds, such as (−)-10-epi-γ-eudesmol, found in geranium oil, have the potential for commercial development.

KEYWORDS: *Lone star tick, Amblyomma americanum, geranium oil, Pelargonium graveolens, (−)-10-epi-γ-eudesmol, natural tick repellent*

INTRODUCTION

In the U.S. and elsewhere, the occurrence and geographical distribution of tick-borne diseases have continued to increase over the past 3 decades. Thousands of Americans are infected each year with tick-borne diseases, such as Lyme disease and Rocky Mountain spotted fever. The principal vector species for the transmission of these diseases are three-host ticks that use a variety of vertebrate hosts associated with forested habitats. Despite recent advances in tick control technology, large-scale reduction of tick populations has not been achieved. Synthetic chemical repellents are a commonly accepted means of personal protection against tick bites.[1] Lone star ticks, *Amblyomma americanum* (L.), have recently risen in importance as a human health risk and are notorious nuisance biters.[2−4] These ticks are active host seekers that are strongly attracted to host-produced CO_2.[5,6]

Synthetic repellents, in particular *N*,*N*-diethyl-meta-toluamide (DEET)-based products that were developed in the 1950s, have been used widely to protect humans from mosquitoes and ticks.[7] Newer synthetic repellents, such as icaridin (picaridin) and the biopesticide IR3535, have also been used against ticks.[7]

Interest in natural arthropod repellents, such as BioUD,[8] a natural ingredient isolated from wild tomato plants and other biological sources, has sparked the investigation of naturally occurring alternatives to synthetic repellents.[9] Although the Food and Drug Administration and the American Academy of Pediatrics affirm the safety of DEET, there is still a wide public concern about the danger of repetitious use of this repellent, partly because DEET has been associated with seizures and encephalopathy in children[10] and also because parents and caregivers are nervous about the possible effects of synthetic chemical repellents on their children's skin.[11]

U.S. military personnel are currently deployed in many countries, including some where diseases transmitted by arthropods present a threat to their operations. Personal protective methods, such as the use of repellents, are important means of reducing exposure of military troops to vector populations.[12] Although DEET is safe for cotton, wool, and nylon fabrics, it can dissolve plastic on eye glasses, watch crystals, and protective mask eyepieces. Some DEET users have an oily and burning sensation when it is applied to the skin (irritant contact dermatitis).[12] Over 62% of 1500 soldiers who responded to a questionnaire urged the Army to obtain a better repellent.[13]

As a result of these concerns, there is a need to identify and develop alternatives to complement the currently available

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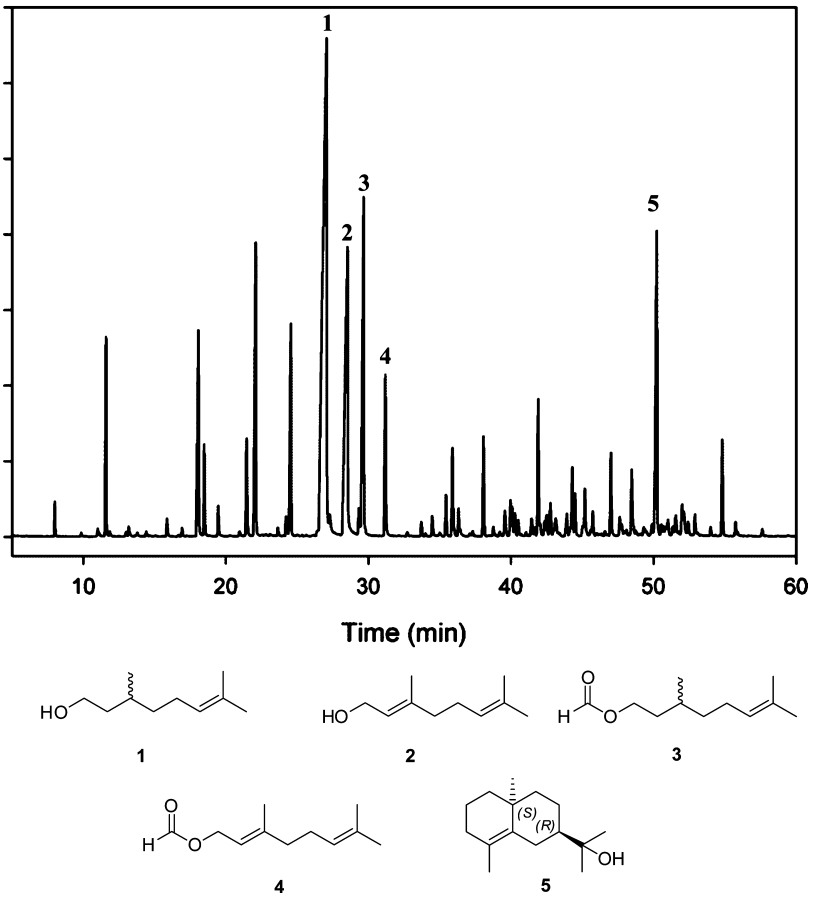

Figure 1. Total ion chromatogram of steam-distilled geranium oil (S1) and its active compounds against *A. americanum*: (1) citronellol, (2) geraniol, (3) citronellyl formate, (4) geranyl formate, and (5) (−)-10-epi-γ-eudesmol.

synthetic repellents for protection against a wide variety of insect-vectored diseases. Although a great deal of information is available about the repellent activity of natural products against mosquitoes, much less data are available regarding tick repellents derived from plant extracts or essential oils. Jaenson et al.[14] found that 30% geranium oil repelled 100% of *Ixodes ricinus* nymphs tested at 2 and 5 min. Although insecticidal and repellent activity of geranium oil against *I. ricinus* has been previously reported,[14,15] there is still no information available about which compounds from the geranium oils are active repellents against ticks. As part of our ongoing interests in novel natural repellents for insect management, we report herein our investigation of commercially available geranium oils by the bioassay-guided isolation and identification of novel bioactive compound(s) in geranium oil that repel *A. americanum* ticks.

MATERIALS AND METHODS

Chemicals. The high-performance liquid chromatography (HPLC)-grade hexane and methylene chloride were purchased from Sigma-Aldrich and Fisher Scientific, respectively. The *n*-alkanes used for the internal standard and the measurement of the retention index were obtained from PolyScience Corporation. DEET (CAS registry number 134-62-3), geraniol (CAS registry number 106-24-1), citronellol (CAS registry number 106-22-9), geranyl formate (CAS registry number 105-86-2), citronellyl formate (CAS registry number 105-85-1), and linalool (CAS registry number 78-70-6) were purchased from Sigma-Aldrich, Inc., St. Louis, MO. (+)-Isomenthone (CAS registry number 1196-31-2) was purchased from Erdogmus Parfum Sanayi, Istanbul, Turkey.

Essential Oils. Two essential oil samples (S14 and S15) were obtained by steam distillation of the in-house authenticated plant materials collected by the NCNPR, University of Mississippi, University, MS. The commercial oil samples (S1−S10) were purchased from different sources. The essential oil samples S1, S4, and S8 originated from Egypt; samples S3, S5, and S7 originated from France; sample S2 originated from China; sample S9 originated from South Africa; and sample S6 originated from China/France/Morocco as per the label. No information is available on the country of origin for oil S10.

Flash Chromatography. Flash chromatography purifications were performed on Biotage Isolera Four (Biotage, Charlotte, NC) using both SNAP and FLASH+ silica gel cartridges. The ultraviolet (UV) detection of the collected fractions was performed at 254 and 220 nm.

Nuclear Magnetic Resonance (NMR). ^{1}H (400 MHz) and ^{13}C (100 MHz) NMR spectra were recorded using an American Varian Mercury plus 400 NMR spectrometer. Chemical shifts were referenced to the residual solvent signal ($CDCl_3$: δ_H 7.26 ppm; δ_C 77.1 ppm). Homonuclear ^{1}H connectivities were determined from two-dimensional (2D) correlation spectroscopy (COSY) experiments. One-bond heteronuclear ^{1}H−^{13}C connectivities were determined from gradient heteronuclear multiple-quantum coherence (HMQC). Two- and three-bond ^{1}H−^{13}C connectivities were determined by gradient heteronuclear multiple-bond correlation (HMBC) optimized for a $^{2,3}J_{C,H}$ of 8 Hz.

Optical Rotation. Optical rotations were measured with the samples dissolved in $CHCl_3$ on a Rudolph Research Analytical digital polarimeter at 20 °C, using a 5 cm path length cell.

Gas Chromatography/Mass Spectrometry (GC/MS). The GC/MS system consisted of an Agilent 7890A gas chromatograph and an Agilent 5975C mass selective detector. The injections were made with an Agilent 7693 autosampler. The system was controlled by

ChemStation software (version E.02). A 30 m × 0.25 mm fused silica capillary GC column coated with a 0.25 μm film of 5% phenyl methylpolysiloxane (HP-5MS) from J&W Scientific was used for the essential oil analysis. The inlet temperature was set at 250 °C, and the injector was operated in a split mode with a split ratio of 25:1. The oven temperature was kept at 50 °C for 5 min, programmed to 200 °C at a rate of 2 °C/min, then programmed to 280 °C at a rate of 8 °C/min, and kept constant at 280 °C for 30 min. The mass spectrometer was operated in a scan mode over a mass range of 50−650 atomic mass units (amu) with the electron impact (EI) voltage at 70 eV.

All of the essential oil samples were diluted in methylene chloride prior to the GC/MS analysis. *n*-Dodecane was used as the internal standard, and all of the reported percentage peak areas were normalized against it. The total ion chromatogram (TIC) of sample S1 is shown in Figure 1. A total of 41 major compounds presented in the oil sample were identified by comparing the retention indices to the reference standards, mass spectra, and National Institute of Standards and Technology (NIST) library searches. The results are given in Table 1. The homologous series of *n*-alkanes (C_9−C_{18}) was used as the standards for the calculation of retention indices.

Isolation of (−)-10-epi-γ-Eudesmol. Fast isolation of (−)-10-epi-γ-eudesmol was performed in two steps using flash chromatography. The essential oil (sample S1) (4.38 g) was loaded on a silica gel samplet and then purified on a SNAP KP-Sil 100 g cartridge using hexane/methylene chloride (50 mL/min, gradient elution from 20 to 60% methylene chloride in 10 column volumes). Elution was monitored online at λ 220 and 254 nm. Seven fractions were thus obtained: A1 (69 mg), A2 (106 mg), A3 (596 mg), A4 (1.14 g), A5 (325 mg), A6 (268 mg), and A7 (708 mg). GC/MS analysis showed the presence of (−)-10-epi-γ-eudesmol in fractions A6 and A7. Fraction A6 was subsequently further purified using silica gel FLASH 25+M KP-Sil Biotage cartridges with hexane/ethyl acetate (gradient from 5 to 20% ethyl acetate in 10 column volumes with a flow rate of 25 mL/min) yielding 38 mg of (−)-10-epi-γ-eudesmol. The eudesmol material was obtained as a colorless oil. Identification of (−)-10-epi-γ-eudesmol was performed by one-dimensional (1D) and 2D NMR, and the data were compared to the literature data.[16,17] $[\alpha]_D^{20} = -49.0$ (c = 0.6 g/100 mL in $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 2.70 (bm, 1H), 2.10 (bm, 1H), 1.89 (bm, 2H), 1.68 (bm, 2H), 1.68 (bs, 3H), 1.67 (m, 1H), 1.66 (m, 1H), 1.58 (m, 1H), 1.51 (m, 1H), 1.39 (m, 1H), 1.32 (m, 1H), 1.28 (m, 1H), 1.24 (s, 3H), 1.18 (s, 3H), 1.08 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 135.1, 126.1, 74.7, 44.2, 39.6, 38.2, 34.6, 32.9, 30.0, 28.0, 26.0, 25.5, 22.7, 19.8, 19.0. Mass spectral peaks were observed at *m*/*z*: 222, 204, 189, 161, 133, 91, and 59.

Ticks. *A. americanum* nymphs were obtained from a colony at Oklahoma State University and held at 23−24 °C, 97% relative humidity (RH), and a photoperiod of 16:8 h (light/dark). The ticks were tested 2−3 months after molting.

Vertical Filter Paper Bioassay. The tendency of host-seeking ticks to climb, especially in the presence of host-produced stimuli (CO_2), was used to test the response of *A. americanum* nymphs to repellent treatments in an *in vitro* bioassay described in detail by Carroll et al.[18] A picture of the bioassay apparatus is shown in the Table of Contents graphic. A 4 × 7 cm rectangle of Whatman no. 4 (20−25 μm) filter paper was marked with a pencil into two 1 × 4 cm zones at the far ends of the paper strip and a central 4 × 5 cm zone (as depicted in the study by Carroll et al.[18]). Using a pipettor, 165 μL of test solution was evenly applied to both sides of the central zone of the filter paper. After allowing 10−15 min for drying, the paper strip was suspended lengthwise by a bulldog clip from an Aptex no. 10 double-clip work holder (Aptex, Bethel, CT). A Petri dish (9 cm diameter) glued in the center of a 15 cm Petri dish created a moat when water was added between their walls. The moated Petri dishes were placed directly beneath the suspended filter paper. When *A. americanum* nymphs climbed to the rim of a storage vial opened in the center of the moated Petri dishes, the paper strip was removed from the work holder and held near the rim of the vial until 10 ticks crawled onto the lower untreated zone. The locations of the ticks were recorded at 1, 3, 5, 10, and 15 min after all 10 *A. americanum* nymphs had climbed onto the lower untreated zone of the filter paper (typically within 90 s). The ticks were considered repelled if they were in the lower untreated zone at 15 min or if they fell from the filter paper without having crossed the upper boundary of the treated zone.

The 10 geranium essential oils and DEET as a positive control were tested against *A. americanum* nymphs at 0.103, 0.052, and 0.026 mg/cm^2 of filter paper and an acetone control. A total of 30 ticks were tested for each oil and DEET concentration (except DEET at 0.026 mg/cm^2, for which 20 ticks were tested), and for acetone controls, n = 120 ticks. Subfractions A4−A7 were tested at 0.103 mg/cm^2 of filter paper against 30 ticks each (for controls, n = 40), and A3 was tested separately at 0.413, 0.206, 0.103, and 0.052 mg/cm^2 of filter paper against 30, 30, 20, and 10 ticks, respectively (for controls, n = 60). Geraniol, citronellol, geranyl formate, and citronellyl formate were

Table 1. Composition of S1 Essential Oil

RI*	compound	area (%)	identification method
917	α-pinene	0.37	a
1037	*cis*-linalool oxide	0.27	b
1070	linalool	3.97	a
1077	*cis*-rose oxide	1.16	a
1091	*trans*-rose oxide	0.46	a
1122	menthone	1.57	a
1132	isomenthone	5.33	a
1164	α-terpineol	0.37	a
1207	citronellol	26.76	a
1210	β-citral	0.69	b
1229	geraniol	10.75	a
1242	α-citral	0.56	b
1246	citronellyl formate	7.34	a
1270	geranyl formate	2.53	a
1320	citronellyl isobutyrate	0.31	a
1334	α-cubebene	0.66	b
1341	β-bourbonene	1.44	b
1348	geranyl acetate	0.55	a
1374	β-caryophyllene	1.55	a
1397	α-gurjunene	0.50	a
1403	isoledene	0.55	b
1405	citronellyl propionate	0.44	b
1408	α-caryophyllene	0.35	b
1426	cadinene	0.35	b
1433	germacrene D	2.31	b
1442	ledene	0.32	b
1446	elixene	0.52	b
1451	α-muurolene	0.45	b
1463	T-cadinene	0.42	b
1469	δ-cadinene	1.26	b
1472	calamenene	0.67	b
1483	citronellyl butyrate	1.02	b
1491	alloaromadendrene oxide	0.41	b
1510	geranyl butyrate	1.33	b
1520	spathulenol	0.29	b
1533	phenethyl tiglate	1.21	b
1559	(−)-10-epi-γ-eudesmol	6.25	a, c
1580	guainene	0.40	b
1586	β-selineol	0.64	b
1600	citronellyl acetate	0.39	b
1629	geranyl tiglate	1.52	a
	total percentage	88.24	

*RI, retention indices calculated against *n*-alkanes (C_9−C_{18}); %: calculated from peak area percentage; a, identification was based on standards from the Natural Products Research, University of Mississippi respository; b, tentatively identified on the basis of computer matching of the mass spectra of peaks with the NIST library; and c, characterized by NMR and optical rotation.

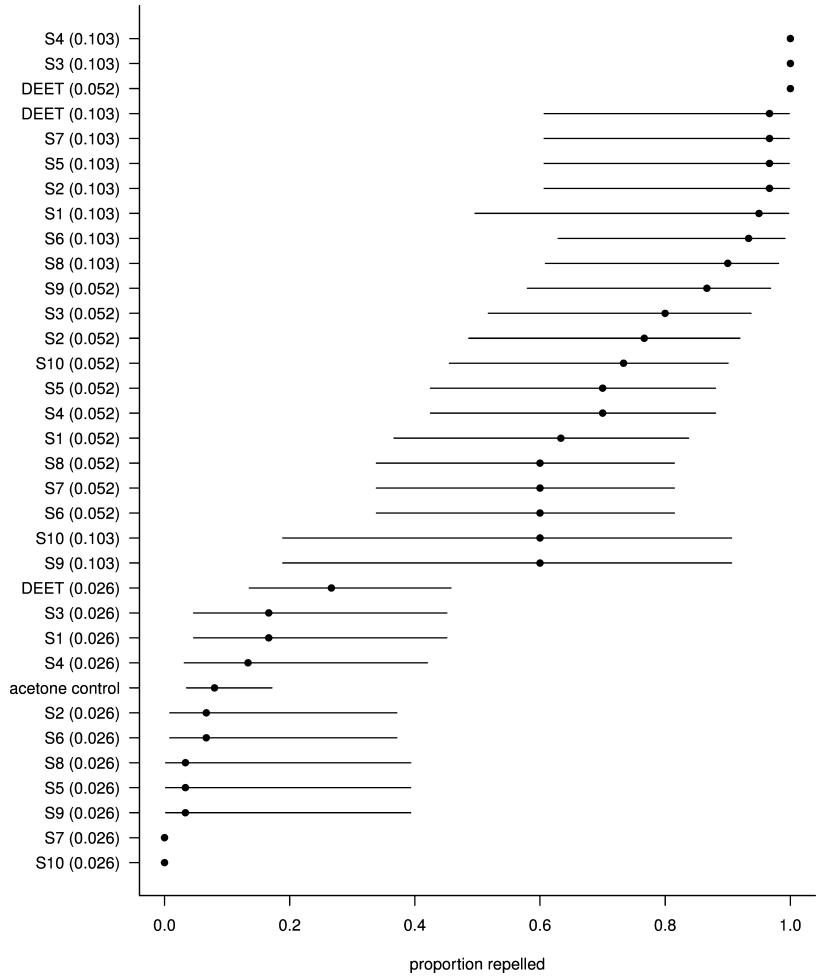

Figure 2. Ranking of geranium essential oils and acetone based on their repellency to *A. americanum* nymphs. Concentrations, as milligrams of oil per centimeter squared of filter paper, of test solutions are in parentheses. Horizontal lines give approximate 95% confidence intervals on the proportion (not calculated for 0 or 100% effective).

tested at 0.413, 0.206, and 0.103 mg of compound/cm^2 of filter paper (20 or 30 ticks per compound/concentration, except 0.103 mg of compound/cm^2 of filter paper, for which n = 10) and an acetone control (n = 150). Linalool was tested at 0.413 mg of compound/cm^2 of filter paper, and isomenthone was tested at 0.103 mg/cm^2 of filter paper. (−)-10-epi-γ-Eudesmol and the repellent DEET were tested at 0.413, 0.206, 0.103, 0.052, 0026, and 0.013 mg/cm^2 of filter paper against 30 ticks for each compound and concentration, except only 10 ticks were tested against DEET at the highest concentration and 20 ticks were tested for (−)-10-epi-γ-eudesmol at 0.013 mg/cm^2 of filter paper (for acetone controls, n = 100).

The data were binomial in nature (each tick was either repelled or not). We modeled the binomial counts with a generalized linear model,[19] using the quasi-binomial family. This models the data as samples from a (possibly over-dispersed) binomial distribution, where the logit of the proportion of repelled ticks is a function of the compound and its concentration (for compounds tested at multiple concentrations) using the glm function in the R stats package.[20] We report (*a priori*) contrasts with the control.

RESULTS AND DISCUSSION

Evaluation of Geranium Oils for Repellent Activity. A total of 10 commercial geranium oils (S1−S10) were evaluated for their repellent activity against *A. americanum* nymphs (Figure 2). Contrasts with controls for oils S1−S10, at a concentration of 0.026 mg/cm^2, produced no significant differences, other than for DEET (over-dispersion parameter = 1.94). However, of oils S1−S10, S3 ranked first or second in repellent activity for each concentration tested. At 0.052 and 0.103 mg/cm^2 (over-dispersion parameters = 1.97 and 1.81, respectively), all oils and DEET differed significantly from controls and repelled >50% of the ticks. Although none of the oils differed significantly from controls at 0.026 mg/cm^2, S1 was chosen for further purification among the active oils because of the adequate quantity available.

Bioactivity of S1 against *A. americanum* Nymphs. A total of 41 compounds were identified by GC/MS analysis in the S1 oil sample, making up 88% of the oil (Table 1). The S1 oil was found to contain citronellol (27%), geraniol (11%), citronellyl formate (7%), and 10-epi-γ-eudesmol (6%) as major constituents. Fractionation of the oil on silica gel gave five major subfractions A3−A7 (Table 2), and all subfractions were subsequently evaluated for tick bioassays. There was overlap in efficacy among the more repellent subfractions, particularly noticeable at 0.103 mg of subfraction/cm^2 of filter paper in Figure 3. Bioassay-guided chemical investigation led to the isolation of (−)-10-epi-γ-eudesmol. The activity of subfractions A6 and A7 containing this sesquiterpene alcohol and the previously reported efficacy of (−)-10-epi-γ-eudesmol[21] isolated from amyris essential oil suggested that it had potential as a repellent against *Aedes aegypti* (L.). While (−)-10-epi-γ-eudesmol has been tested against mosquitos,[21] in the current

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Table 2. Major Compounds Identified in Subfractions (A3–A7) from the S1 Essential Oil

subfractions	A3		A4		A5		A6		A7	
major compounds identified	compound	area (%)	compound	area (%)	compound	area (%)	compound	area (%)	compound	area (%)
	rose oxide	1.7	linalool	11.0	β-bourbonene	1.8	linalool	7.0	linalool	29.5
	isomenthone	9.4	citronellol	72.9	β-caryophyllene	19.6	isomenthone	51.8	citronellol	22.1
	menthone	6.3	geraniol	14.4	α- muurolene	14.5	(−)-10-epi-γ-eudesmol	49.9	(−)-10-epi-γ-eudesmol	34.4
	citronellyl formate	42.5			δ-cadinene	28.9				
	geranyl formate	18.3								

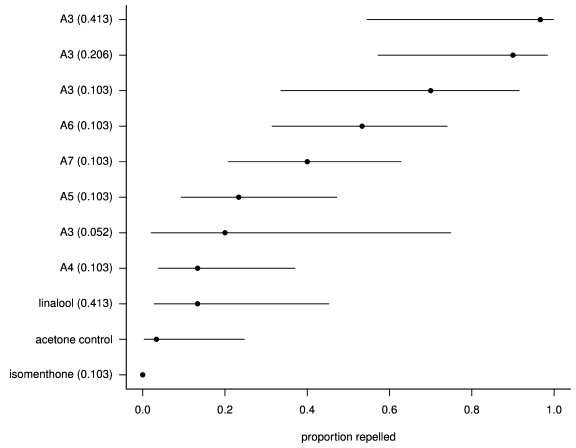

Figure 3. Proportion of ticks repelled (points) by subfractions of geranium oils S1 (concentrations in parentheses, in mg/cm^2), linalool, and isomenthone, ordered by their ranking in effectiveness. Horizontal lines give approximate 95% confidence intervals on the proportion (not calculated for 0% effective). Isomenthone, which was only tested at a single low concentration, had lower repellency (no ticks repelled) than the acetone control.

study, (−)-10-epi-γ-eudesmol was tested for the first time against *A. americanum* nymphs. (−)-10-epi-γ-Eudesmol was previously reported as a marker compound for *Pelargonium graveolens* cultivars from Egypt.[22] The natural presence of (−)-10-epi-γ-eudesmol in some authenticated geranium cultivars has been confirmed by GC/MS analysis (samples S14 and S15; data not shown).

Few research groups have described the isolation of (−)-10-epi-γ-eudesmol.[16,23] Previous isolation procedures reported the purification of this sesquiterpene alcohol (mainly from natural sources other than geranium) using chromatography with either silica gel or silver-nitrate-impregnated silica gel. The latter method is known to produce a large variety of byproducts, and the reproducibility is very dependent upon the technical skills of the operator.[24] In the current study, (−)-10-epi-γ-eudesmol from the complex essential oil of *P. graveolens* was isolated using a fast and highly reproducible method. The purification was performed in just two steps using a gradient of hexane/methylene chloride followed by a second step using hexane/EtOAc. The method is suitable for scale-up and gave acceptable separation even from the sesquiterpene alcohol linalool, which showed a very similar polarity profile with (−)-10-epi-γ-eudesmol. The spectroscopic and optical rotation data were consistent with those reported for (−)-10-epi-γ-eudesmol, and the compound is known to be one of the major constituents in some varieties of *P. graveolens*. However, surprisingly, its enantiomer, 7-epi-γ-eudesmol, has also been reported in the literature as levorotatory.[25]

In addition to (−)-10-epi-γ-eudesmol, linalool, isomenthone, geraniol, citronellol, geranyl formate, and citronellyl formate were found in other subfractions; these compounds were also tested in pure form for tick repellent activity. Five of these compounds [geraniol, citronellol, geranyl formate, citronellyl formate, and (−)-10-epi-γ-eudesmol] constituted in total 53.6% of sample S1. All five strongly repelled *A. americanum* nymphs at a concentration of 0.206 mg/cm^2 and higher (Figure 4). The most repellent of these was (−)-10-epi-γ-eudesmol, which at 0.103 and 0.052 mg of compound/cm^2 of filter paper, repelled 90 and 73% of the ticks, respectively. As shown in Figure 4, at

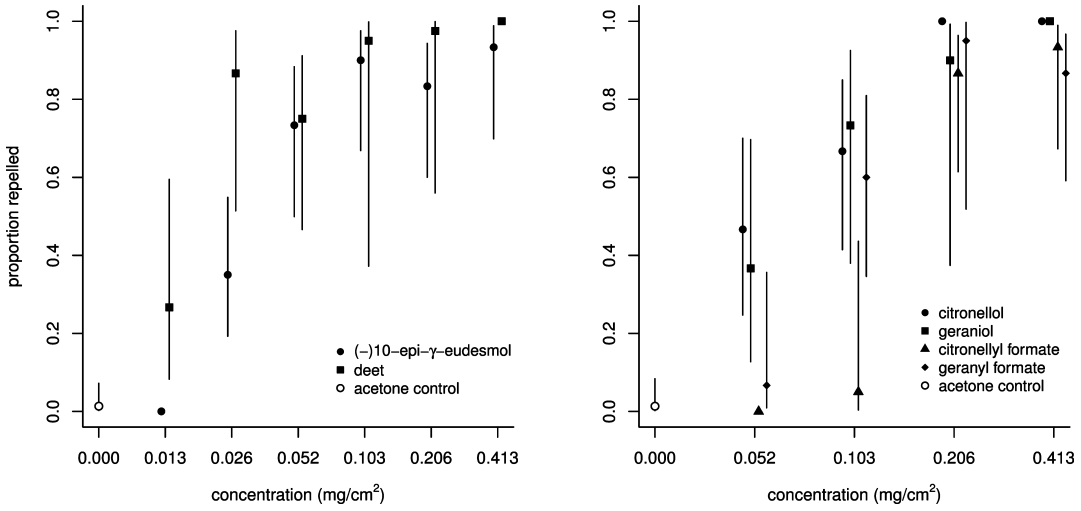

Figure 4. Proportion of ticks repelled at various concentrations of (left panel) (−)-10-epi-γ-eudesmol and DEET and (right panel) citronellol, geraniol, citronellyl formate, and geranyl formate in an acetone solvent. Back-transformed 95% confidence intervals (for proportions other than 0 and 1) were calculated from a generalized linear model with a logit link. Estimates include an over-dispersion parameter.

higher concentrations, (−)-10-epi-γ-eudesmol and DEET were similarly repellent to *A. americanum* nymphs.

On the basis of bioassay behaviors, Weldon et al.[26] ranked geraniol and citronellol as the two most repellent of the 24 compounds occurring in citrus that they tested at a single concentration (3 μL of 0.1 M solution/cm^2) against *A. americanum*. We found that geraniol and citronellol repelled 90% (n = 20) and 100% (n = 30), respectively, of the ticks at 0.206 mg/cm^2 of filter paper, but at 0.052 mg/cm^2, fewer than half of the ticks were repelled by any of the pure compounds. Geranyl formate and citronellyl formate were similarly effective, 95% (n = 20) and 86.7% (n = 30) at 0.206 mg/cm^2 of filter paper and, likewise, fell below 50% repelled when the concentration was reduced to 0.052 mg/cm^2. Citronellol and geraniol are also reported to repel nymphs of the sheep tick, *I. ricinus* L.[27−29] The cattle tick, *Rhipicephalus appendiculatus* Neumann, was repelled by geraniol[30] and α-terpineol.[31] In our study, linalool and isomenthone were ineffective at the concentrations tested (Figure 3). Lwande et al.[30] found linalool to be moderately repellent to *R. appendiculatus*. Tunón et al.[28] reported some repellent activity for linalool against *I. ricinus*, but Del Fabbro and Nazzi[32] reported that linalool failed to repel *I. ricinus*. Lesser amounts of α-terpineol, citral, and citronellyl acetate, previously reported repellents of *A. americanum*,[26,28] were also present in the sample S1. It should be kept in mind that plant-produced chemicals that deter invertebrates and vertebrates primarily target herbivores and not blood feeders, such as ticks. Probably because of their shared arthropod lineage with herbivorous insects, ticks may also be susceptible to some plant-produced deterrents.

In conclusion, we report here for the first time the biological activity of (−)-10-epi-γ-eudesmol as a tick repellent along with a feasible method for fast purification and scale-up. Our bioassay-guided investigation showed (−)-10-epi-γ-eudesmol to be an effective repellent against *A. americanum*. The efficacy of (−)-10-epi-γ-eudesmol was similar to that of DEET at concentrations of ≥0.052 mg of compound/cm^2 of filter paper. At lower concentrations, (−)-10-epi-γ-eudesmol lost much of its activity, whereas the activity of DEET did not decline until 0.013 mg of compound/cm^2 of filter paper. On the basis of the observed strong repellent activity of (−)-10-epi-γ-eudesmol against *A. americanum*, this compound may be a useful component of natural repellent-based formulations. Many commercial repellent products contain well in excess of 5% of active ingredients. Current results show that (−)-10-epi-γ-eudesmol retains repellent activity even when present in less than 5% as an active ingredient, and it can be expected that a potential repellent product containing ≥20% (−)-10-epi-γ-eudesmol should provide good protection against tick bites.

■ ASSOCIATED CONTENT

Supporting Information

One- and two-dimensional NMR data along with the EI−MS spectrum of (−)-10-epi-γ-eudesmol. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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