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Research Article

Structure-Repellence Potential of Stereoisomers of Menthane-Diol and Analogues against the Brown Ear tick (*Rhipicephalus appendiculatus*)

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Abstract

Tick-borne infections in livestock are wide-spread in Africa. They present a great constraint to livestock development, particularly in the improvement of local breeds. This is due to the fact that many different tick-borne infections occur in the continent. This problem is magnified by the high susceptibility of foreign breeds of livestock being used to improve livestock productivity in many African countries. Brown Ear tick, *Rhipicephalus appendiculatus*, is the vector of *Theileria parva*, the pathogen of the tick–borne disease, East Coast Fever. A commonly used conventional arthropod repellent N, N-diethyl-3-methylbenzamide (DEET) is still used for repelling a wide range of insects such as, ticks and mites by many famers in Kenya. However, conventional drugs have many harmful effects to both the animals and human beings, additionally they cause considerable environmental pollution. In search for effective green and non-toxic alternatives to DEET against different hematophagous arthropods, there has been renewed interest in repellents of botanical origin. Phytochemicals have arguably fewer side effects and are readily available. A monoterpene of relatively low volatility, p-menthane-3,8-diol (PMD), obtained from lemon eucalyptus leaves (*Eucalyptus citriodora*) has shown potent repellence against mosquitoes. This study was designed to evaluate the structure-activity studies of p-menthane-3,8-diol stereoisomers and analogues against the Brown ear tick. The essential oil of lemon eucalyptus was extracted by hydrodistillation. Commercial standards of (+) and (-)-isopulegol were hydrated at C-8 using the oxy-mercuration/demercuration procedure to obtain (+) and (–)-trans-p-menthane-3,8-diol respectively. (±)-Cis-p-menthane-3,8-diol stereoisomers were prepared from (±)-citronellal via the *Zimmermann* and English procedure that involved acid catalyzed cyclization of (±)-citronellal. GC-MS was used to identify the chemical composition of E. citriodora oil, while the structural elucidation of the synthesized PMD stereoisomers was done using 1H-NMR and 13C-NMR spectroscopy. The *E. citriodora* oil, menthane diol stereoisomers, its analogues and DEET were screened for their repellent activity against *R. appendiculatus*, through subjecting them to a dual choice tick climbing bioassay. The results revealed that methane diols were potently repellent against *R. appendiculatus* and comparable to that of DEET. Racemates of cis and trans were as repellent as (+) and (–)-trans diols. PMD analogues of the diol (L-menthol, 1-α-terpineol) showed much lower repellency against R. appendiculatus compared to p-menthane-3,8-diol stereoisomers. *E. citriodora* oil had much lower repellency than PMD stereoisomers, but significantly higher repellency than L-menthol and 1-α-terpineol. This study concludes that menthane-diol stereoisomers and E. citriodora oil have potent repellency activity against the Brown Ear tick (*Rhipicephalus appendiculatus*) and can be used in the management of ticks and insects in livestock.

Keywords: *Eucalyptus citriodora*; *Rhipicephalus appendiculatus*; p-menthane-3; 8-diol stereoisomers; repellency

Introduction

In sub-Sahara Africa, East Coast Fever (ECF) is caused by *Theileria parva* [1] and transmitted by Brown ear tick, *Rhipicephalus appendiculatus*. ECF is one of the major constraints to the development of the livestock industry [2] An estimated 9.65 million heads of cattle (both indigenous and exotic) are at risk of ECF [3]. The disease is associated with up to 10% mortality in zebu calves in ECF endemic areas and can cause up to 100% mortality in susceptible exotic and indigenous breeds [4].

Control and management of both vector and pathogen have continued to rely heavily on the application of synthetic chemical acaricides on the host. This has proved to be unsuitable in many ways [5]. The acaricides can eliminate ticks from the host, but do not prevent continued re-infections from the source environment where ticks spend 90% of their life. For effective management of ticks, there is need to look for a mechanism to control ticks on individual hosts as well as in the host environment in order to control re-infections during grazing. One possible strategy towards achieving this would be use of tick's repellents on the host and tick-repellent plants in the pasture [6].

A commonly used commercial arthropod repellent, *N,N*-diethyl-3-methylbenzamide (DEET), is still considered the best available product repelling a wide variety of insects, ticks and mites [7]. In humans, however, the repellent may cause insomnia, mood disturbances, impaired cognitive functions, seizures, toxic encephalopathy and allergic reactions [9]. Though DEET is not expected to be bio-accumulative, it has been found to cause considerable environmental pollution [8]. More recent research has shown that mosquitoes, as well as being 'blinded' by the chemical, actively dislike the smell of DEET [8]. Studies have also shown that plant-based repellents can be as effective as DEET or even better [9]. The practical application of tick-repellent plants and essential oils and their integration with other measures either on the host or in the pasture land could be a practical and economical way of controlling not only livestock ticks but other arthropod vectors [10].

p-Menthane-3,8-diol, obtained from lemon eucalyptus (*Eucalyptus citriodora)*, known for a long time in China for its mosquito repellent properties, has shown particular promise in this regard [11]. The essential oil of *E. citriodora*, with its high content of citronellal, is one of the three perfumery oils distilled on commercial scale from the *eucalyptus* species [12]. In addition to yielding an important essential oil from its leaves, *E. citriodora* is grown for its sawn timber. The wood has also been reported to be resistant to termite attack [13]. It has long been shown that oil of lemon Eucalyptus has repellent effects on mosquitoes and chemical studies have shown that its principal constituents are citronellal, isopulegol and α-pinene [14].

Materials and Methods

In this study, *p*-menthane-3,8-diol stereoisomers were synthesized from isopulegol and citronellal. All synthetic chemicals and DEET were purchased from Sigma - Aldrich Chemical Company (USA) with a purity of 95%.

(+) and (−)-isopulegol were hydrated at C-8 using the oxy-mercuration/demercuration procedure to obtain (+) and (–)-*trans*-*p*-menthane-3,8-diol respectively. (±)-*Cis*-*p*-menthane-3,8-diol and (±)-*trans*-*p*-menthane-3,8-diol stereoisomers were prepared from (±)-citronellal via the Zimmermann and English procedure that involved acid catalyzed cyclization of (±)-citronellal. The current study also sought to establish if there exists any stereochemical requirement(s) for repellent action of *p*-menthane-3,8-diol against the *R. appendiculatus*, and how the blend of isomers (racemic mixture) compares with its single isomer. To establish the structural requirements for repellency of *p*-menthane-3,8-diol against the *R. appendiculatus*; two analogues of PMD were bio-assayed, 1-α-terpineol (hydroxyl group at C-8, with a double bond at C-2) and L-menthol (hydroxyl group at C-8). *Experimental Ticks*, *R. appendiculatus* were obtained from colonies at the International Livestock Research Institute (ILRI) and bred at *International Centre of Insect Physiology and Ecology* ICIPE, Nairobi County in Kenya. Rearing conditions were as described by [14].

Tick repellency Bioassay

The tick climbing bioassay design exploited the well-known predisposition of ticks to climb up and aggregate on grass stem to await passing host [16]. This experiment was set up at ICIPE, Nairobi County, Kenya. An aluminum base of area 105 cm^2 with two stands of 26 cm in height and 7.0 cm apart was put in a basin of water, 1.5 cm deep (the water restricts the movement of the ticks to the aluminum base). Two sets of glass tubes were used; one of 4.5 cm (outer one) and the other one 0.8 cm (smaller inner tube) in diameter. A strip of filter paper (Whatmann No 7, 2 cm wide) was stapled to form a collar around the upper parts of each smaller inner glass tubes at a distance of 15 cm from the aluminum base to provide the source of either test odors or pure solvent. A set up consisting of an aluminum base (15 × 7 × 1.5 cm) with a pair of aluminum rods (26 cm l × 0.7 cm d) 7 cm apart covered with glass tubes (0.8 cm d) was used (Figure 1). One collar on the pair of tubes was treated with test sample solution and the other with the solvent (dichloromethane) to serve as control. After the solvent was allowed to evaporate (10 min), these tubes were shielded with wider tubes (4.5 cm d) from 4 cm above the aluminum base to facilitate relatively uniform vertical gradients of the test sample along the 3.7 cm gap between two tubes. Wet cotton wool plugs on the top of these tubes ensured relatively high humidity (>75 %) within the columns. Ten ticks of mixed age and sex were placed at the Centre of the aluminum base and observed for 60 minutes. The apparatus was placed in a tray with shallow water, which prevented the dispersal of test ticks from the base.

Initial comparison of the responses of the ticks in the set up with and without residual dichloromethane on one and both sides, showed no bias for either side and no effects of the residual solvent. A piece of whatman paper measuring 4 cm by 1 cm, folded and stapled to make a ring, was impregnated and placed on the glass covering about 12 cm from the bottom. The test materials (synthesized *p*-menthane-3,8-diol stereoisomers, analogues, *Eucalyptus citriodora* oil and the aqueous fraction) and the solvent were dispensed using a calibrated Eppendorf pipette and equilibrated for 30 min before ten adult ticks of mixed ages and sexes were released at the centre of the aluminum base. Prior to each bioassay, ticks were kept at high relative humidity (>85% RH) for 24 hrs in containers with moist cotton wool, so that they were not dehydrated and as a result would have less tendency to drown in the water surrounding the aluminum base (Figure 1). All bioassays were conducted in a room kept at 28 ± 1 °C and 75 ± 5% RH, which had an exhaust fan running continuously. The assays were run for 60 minutes, and the number of ticks above the filter paper strip on the control glass tube (Nc) and on the treated glass tube (Nt) were recorded at 15, 30, 45 and 60 minutes. After each test, the apparatus was thoroughly cleaned and dried at 100 °C. Each dose of the test material was tested 12 times; each time with a fresh, naive adult tick. The repellent effect of the essential oils was evaluated according to the formula adopted by [17] on equation 1.0 below.

Where Nt and Nc represent the number of ticks that climbed on or passed the treated and control collar of filter papers on the glass tubes, respectively.

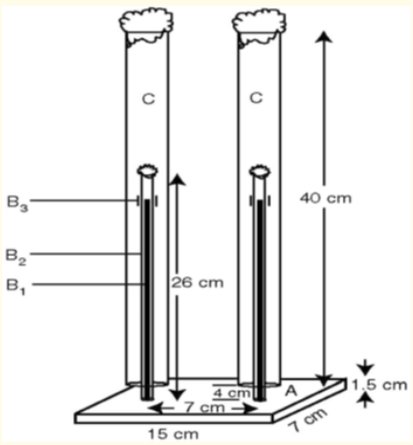


Figure 1: Tick climbing bioassay apparatus [15].

Tick climbing bioassay apparatus (placed in a tray with shallow water, not shown): A, aluminium base; B_1, aluminium rod (26 cm l × 0.7 cm d); B_2, 0.8 cm d glass tube; B_3, filter paper collar; C, 4.5 cm d glass tube plugged with dry cotton wool. The two aluminium rods, B_1 on the aluminium base, A, (15 x 7cm), were 7 cm apart. The outer tubes, C are held in position, 4 cm above the aluminium base, A by a retort stand clamp. The ten ticks were introduced on the aluminium base, A, at a position marked with a star, 3.5 cm from the base of the aluminium rods, B_1 [17].

Synthesis of (+)- and (–)-trans-p-menthane-3,8-diol from (+)- and (–)-isopulegol.

This synthesis was achieved according to [19]. Mercury (II) acetate (1.3g, 3.9×10^{-3}moles) was weighed; 9ml of water was then added and stirred until the acetate dissolved. Tetrahydrofuran, 9ml was then run in rapidly; followed by (+)- and (−)-isopulegol (0.5g, 3.24×10^{-3} moles). The mixture was stirred at room temperature for 24 hrs to ensure completion of the oxy-mercuration step. Next, 5 mL of 3M NaOH followed by $NaBH_4$ 0.16 g, 4.23×10^{-3}moles of 3.5 b mL NaOH was added slowly. The rate of addition of both solutions was controlled to maintain temperature at 25°C, through cooling. The organic (THF) layer was separated from the aqueous alkaline layer. The aqueous layer was saturated with NaCl and extracted with tetrahydrofuran (3 × 10 ml) to extract the organic compound, dried (anhydrous Na_2SO_4) and filtered. The solvent was removed from the filtrate by evaporating in vacuo at 45°C; further concentration was done by blowing the solvent off under white spot nitrogen, to obtain a white thick opaque liquid. This crude PMD was purified on a silica gel column (230 – 240 mesh); eluent 20-40% ethyl acetate in hexane). Further purification of the PMD product was achieved by crystallization from the hexane-ethyl acetate mixture to obtain the desired product in 90% yield. Structure confirmation was done using ^{1}H-NMR and ^{13}C-NMR spectroscopy.

Synthesis of (±)-cis-p-menthane-3,8-diol and (±)-trans-p-menthane-3,8-diol from (±)-citronellal

This was prepared from (±)-citronellal using the modified procedure of [19]. (±)-Citronellal (2 g, 1.296×10^{-2} moles) was added in a dilute sulfuric acid solution (100 ml, 5%) and stirred for 25 hrs. The organic layer was separated from the aqueous layer. To quench the residual acid sodium bicarbonate was added to the organic layer until the effervescence stopped. The aqueous layer was extracted with dichloromethane (5×20 ml), after saturating the solution with NaCl. The dichloromethane extract was dried using anhydrous Na_2SO_4 and filtered. The solvent was then removed by evaporating in vacuo at 40°C. Purification was done on a silica column (230-240 mesh, eluent 20-40% ethyl acetate in hexane) to separate the target *cis*-diols from the *trans* products and un-reacted starting material. The diol products were analyzed by GC-MS, two sharp peaks at t_r 14.0 and 17.1 minutes were obtained. Separation of the target *cis*-diols from the *trans*-diols was done by preparative HPLC followed by crystallization from the hexane-ethyl acetate to obtain the desired product in 81% yield. Structure confirmation was done using ^{1}H-NMR and ^{13}C-NMR spectroscopy.

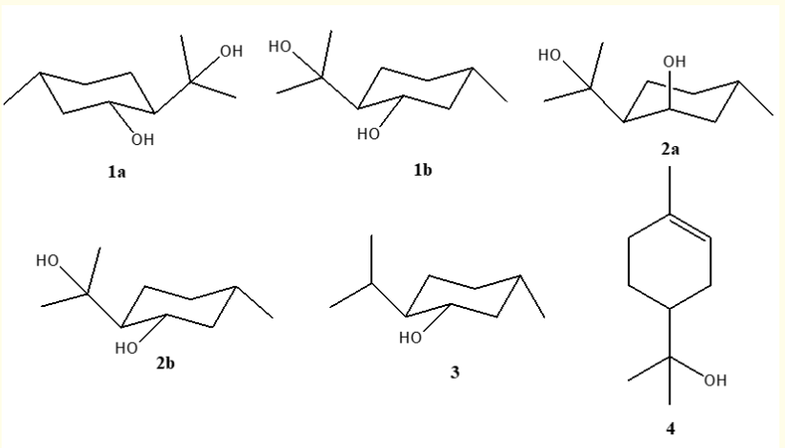


Figure 2: Structures of (+)-trans-p-menthane-3,8-diol (1a); (-)-trans-p-menthane-3,8-diol (1b); (±)-cis-p-menthane-3,8-diol (2a); (±)-trans-p-menthane-3,8-diol (2b); L-menthol (3) and 1-α-terpineol (4).

Gas Chromatography

Mass Spectrometry Structure determination of the components in the *E. citriodora* oil, the synthesized *p*-menthane-3,8-diol stereoisomers were identified using GC-MS. GC-MS analyses were performed with a VG Masslab 12-250 quadruple gas chromatography-mass spectrometer. Chromatographic separations were achieved using a fused silica capillary column (Hewlett Packard, 50 m x 0.32 mm ID) coated with Carbowax 20M (0.3 µm film thickness) with helium as the carrier gas. Injections were made in the splitless mode with helium as the carrier gas. Compounds were identified by their electron impact (El) mass spectral data, order of elution and relative GC retention times, and by comparison of their mass spectra and GC retention times to those of authentic samples. The computer on the GC-MS system records a mass spectrum for each scan for the unknown chemical in the sample and compares the mass spectrum from a sample component with mass spectra in the National Institute of Standards and Technology (NIST). mass spectral library. Identification of compounds in *E. citriodora* oil and synthesized *p*-menthane-3,8-diol were verified by comparison with authentic samples.

NMR Spectroscopy Structure confirmation of synthesized *p*-menthane-3,8-diol stereoisomers was done using ^{1}H NMR and ^{13}C NMR spectroscopy. ^{1}H NMR (400 MHz) and ^{13}C NMR spectra (101 MHz) were recorded on Agilent NMR spectrometers. The chemical shifts were reported in parts per million (ppm), and the residual solvent peak was used as an internal reference: proton (chloroform δ 7.26) and carbon (chloroform δ 77.0). Multiplicities were indicated as follows: s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), dd (doublet of doublet), br s (broad singlet). Coupling constants were reported in hertz (Hz).

Statistical Analysis The mean % repellency presented in tables of results was computed from the original untransformed data using (R – software, version 2.15.2 (2012-10-26)). Data were normalized by logarithmic (log (n + 1)) transformation before being subjected to one-way analysis of variance (ANOVA). Means between treatments were separated using the Student-Newman – Keuls test at P≤0.05 with SAS. During analysis, percentage repellency (PR) values were converted to repellency probabilities ranging from 0 to 1 in order to fit into a probit model. Dose-response data was subjected probit analyses using the % repellency values obtained from triplicate experiments, and expressed as repellent doses (RD) at RD_{75} using SAS statistical software.

Results

Composition of Eucalyptus citriodora oil Air-dried *E. citriodora* leaves (1 kg) yielded 1.84% (18.4g) of essential oil. Gas chromatography-mass spectrometry (GC-MS) showed the presence of thirty-eight (38) compounds in the oil fraction as shown in figure 3 and table 1. Previously, chemical investigations by [20] had shown that the three principal components of the oil of the Kenyan grown *E. citriodora* are citronellal (65-88%), citronellol (2-25%) and isopulegol (2-19%). However, in this study the principal components of the oil of *E. citriodora* sampled from Arboretum, Nairobi County were citronellal (32.03%), citronellol (19.41%), cineole (9.87%) and isopulegol (5.97%) (Figure 3 and Table 1).

Figure 4-8 shows the total ion chromatogram of the synthesized Menthane-diol stereoisomers. The figures confirm the purity of the synthesized PMD stereoisomers. Figure 7 and 8 was obtained following resolution of racemic, (±)-*cis*-*p*-menthane-3,8-diol (Figure 6), using Chiral Prep-HPLC.

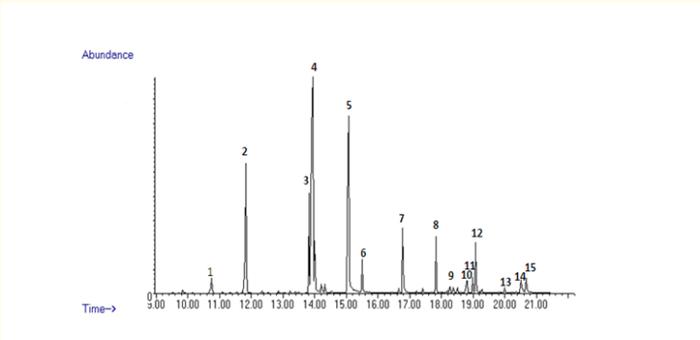
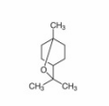
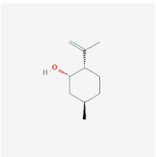
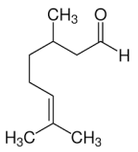
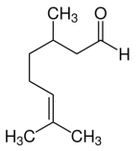
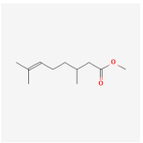
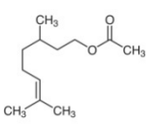


Figure 3: Total ion chromatogram showing *E. citriodora* oil profile.

Peak No.	RT	Area %	Compound Name	Chemical Structure	Relative Abundance (%)
1	10.75	1.32	β-Pinene		1.32
2	11.82	9.87	1,8-Cineole		9.87
3	13.84	5.92	Neo-isopulegol		5.92
4	13.95	32.03	Citronellal		32.03
5	15.07	19.41	Citronellol		19.41
6	15.52	2.32	Methyl citronellate		2.32
7	16.79	5.07	Citronellyl acetate		5.07
8	17.82	2.96	Caryophyllene (E-)		2.96
9	18.27	0.77	α-Humulene		0.77

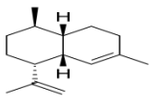
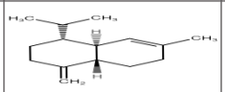
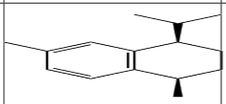
10	18.81	1.59	Amorpha-4,7(11)-diene		1.59
11	19.01	0.92	γ-Cadinene		0.92
12	19.10	2.99	*Cis*-calamenene		2.99
13	20.51	1.48	Dauca-5,8-diene		1.48
14	20.67	1.39	γ-Cadinene		1.39
15	20.83	0.82	*Trans*-dauca-4(11),7-diene		0.82

Table 1: Major constituents in the essential oil of *E. citriodora* analyzed by Gas Chromatography-Mass Spectrometry (GC-MS).

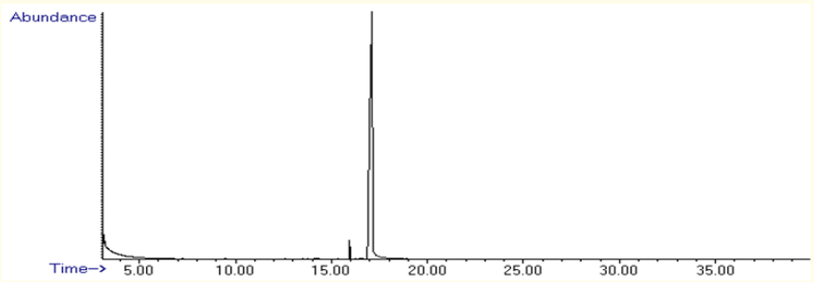


Figure 4: Total ion chromatogram of (+)-trans-p-menthane-3,8-diol synthesised from (+) – isopulegol.

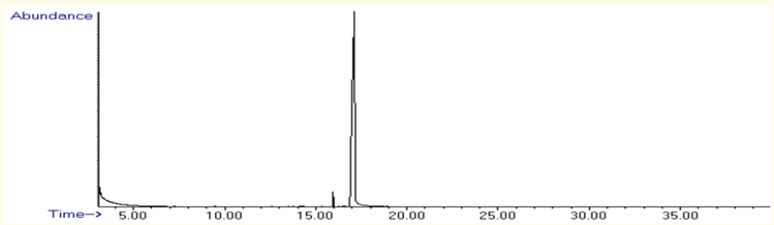


Figure 5: Total ion chromatogram of (−)-trans-p-menthane-3,8-diol synthesised from (−) - isopulegol.

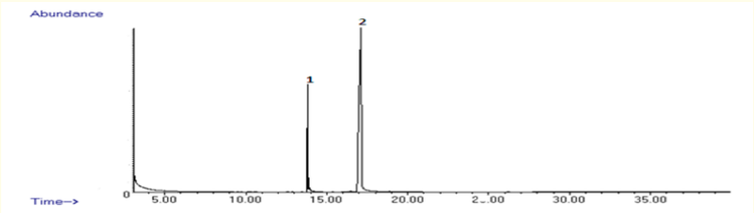


Figure 6: Total ion chromatogram showing (±)-cis-p-menthane-3,8-diol (1) and (±)-trans-p-menthane-3,8-diol (2) profile obtained from (±)-citronellal synthesis.

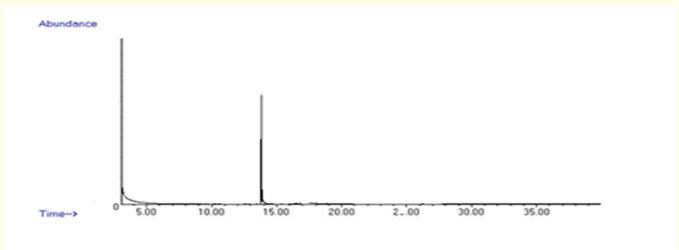


Figure 7: Total ion chromatogram of (cis)-p-menthane-3,8-diol separated by Chiral prep HPLC.

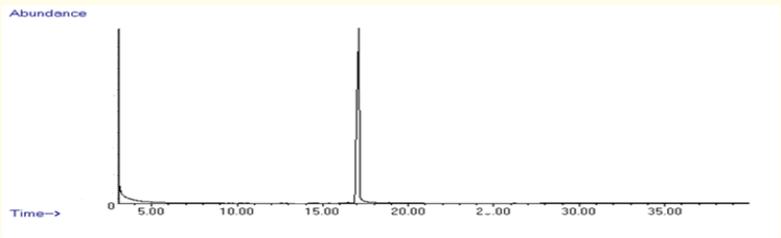


Figure 8: Total ion chromatogram of (trans)-p-menthane-3,8-diol separated by Chiral prep HPLC.

(*Trans*)-*p*-Menthane-3,8-diol gave the following physical and spectroscopic characteristic's m.p 73 -74 °C, $[\alpha]_D$=+10.2 and -9.8 ^{1}H-NMR (400 MHz, $CDCl_3$) : 0.85 - 0.98 (m, 2 H), 0.92 (d, *J*=6.5 Hz, 3H), 1.01 - 1.12 (m, 1 H), 1.16 - 1.25 (m, 6 H), 1.33 - 1.48 (m, 2 H), 1.61 - 1.73 (m, 2 H), 1.89 - 1.98 (m, 1 H), 3.71 (td, *J*=10.50, 4.15 Hz, 1 H), 4.23 (br,s, 1 H) 4.44 (br,s, 1 H). ^{13}C-NMR (100 MHz, $CDCl_3$): 21.93, 23.61, 27.00, 29.92, 31.31, 34.49, 44.41, 53.27, 72.83, 74.97.

(*Cis*)-*p*-Menthane-3,8-diol gave the following physical and spectroscopic characteristic's m.p. 67.5 - 68.0 °C, $[\alpha]_D$=+9.6 and -9.1. *^{1}H*-NMR (400 MHz, $CDCl_3$: 0.87 (d, *J*=6.35 Hz, 3 H), 0.89 - 0.94 (m, 1 H), 1.05 (ddd, *J*=14.53, 12.70, 2.08 Hz, 1 H), 1.12 - 1.18 (m, 1 H) 1.22 (s, 3 H), 1.35 (s, 3 H), 1.63 - 1.75 (m, 2 H), 1.75 - 1.88 (m, 3 H), 3.14 (s, 1 H), 3.41 (br,s, 1 H), 4.40 (br,s, 1 H). ^{13}C-NMR (100 MHz, $CDCl_3$): 20.21, 22.15, 25.52, 28.71, 28.86, 34.80, 42.44, 48.20, 67.96, 73.19.

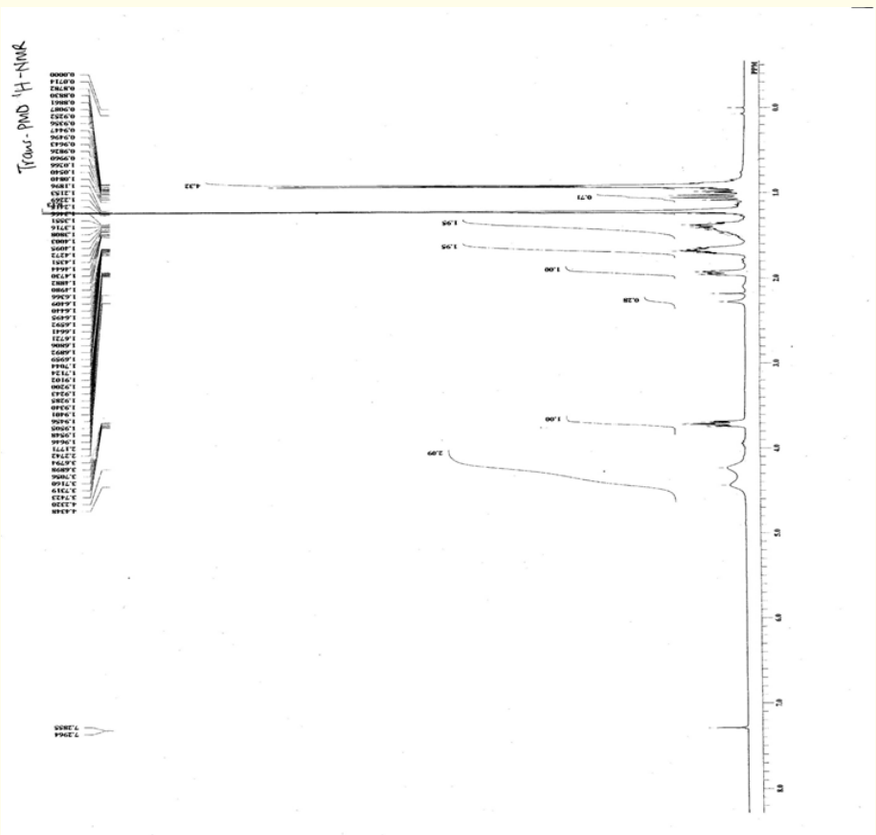


Figure 9: ^{1}H-NMR spectrum of (*trans*)-p-Menthane-3,8-diol.

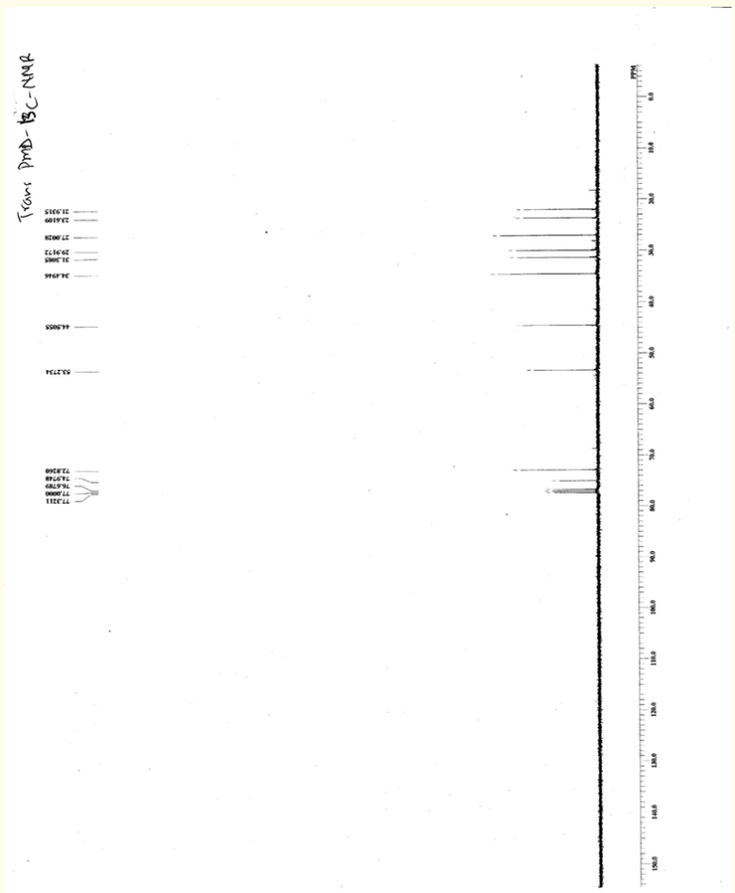


Figure 10: ^{13}C-NMR spectrum of (*trans*)-p-Menthane-3,8-diol.

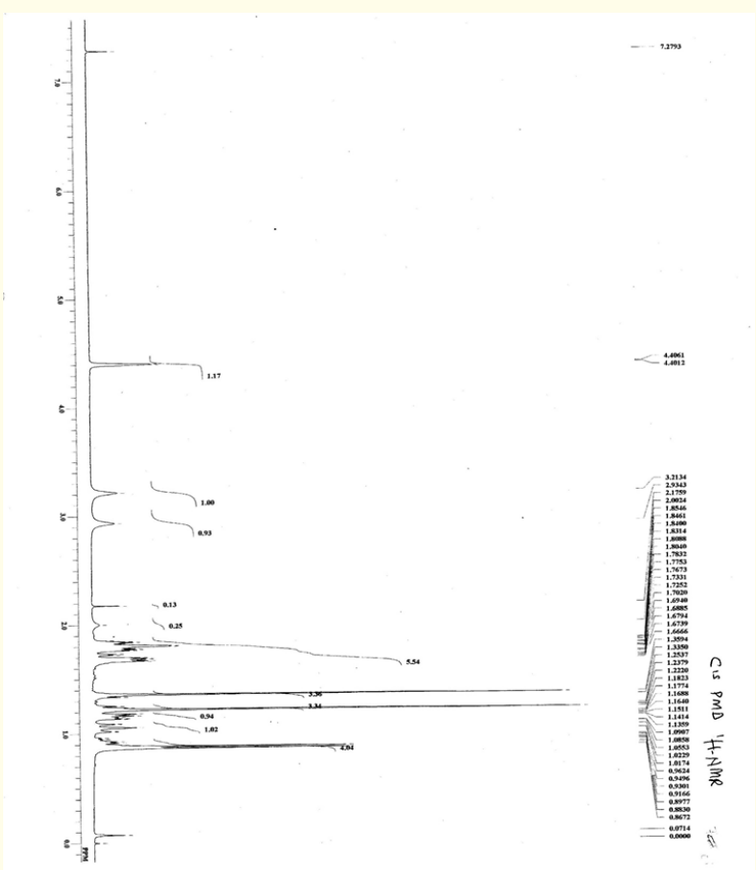


Figure 11: ^{1}H-NMR spectrum of (*cis*)-p-Menthane-3,8-diol.

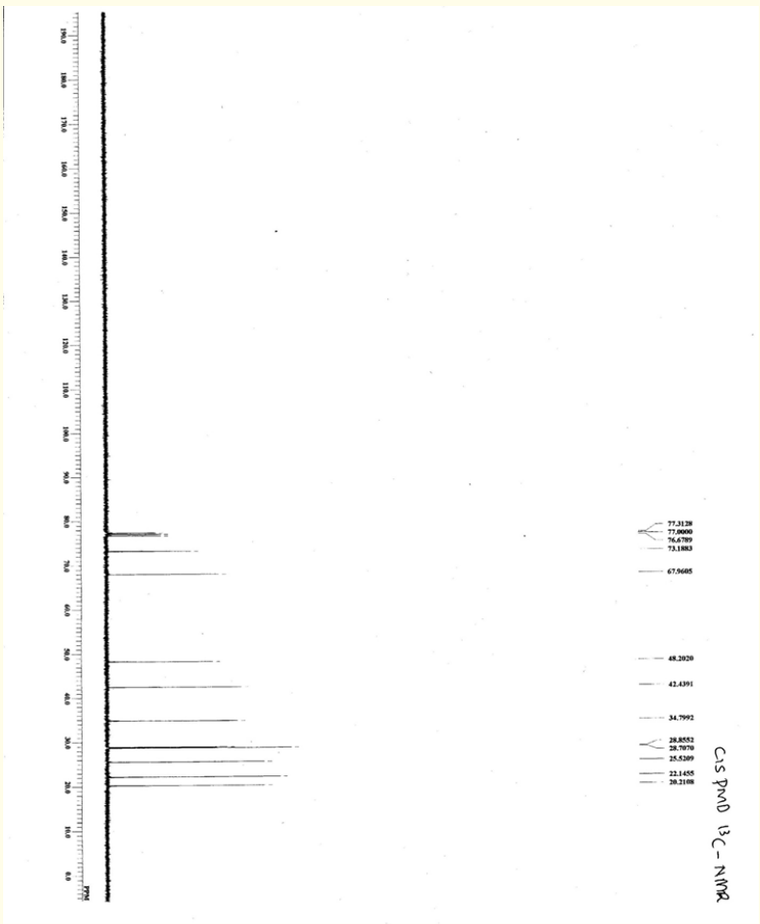


Figure 12: ^{13}C-NMR spectrum of (*cis*)-p-Menthane-3,8-diol.

Abbreviations are as follows: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; br, broad.

Repellency of PMD stereoisomers, analogues, E. citriodora oil, DEET and aqueous fraction

Table 2 provides results of percentage repellency of PMD stereoisomers and DEET, and table 3 provides those of PMD analogues, *E. citriodora* oil and the aqueous fraction of the hydro-distillate. Table 4 summarizes RD_{75} of *p*-menthane-3,8-diol stereoisomers, PMD analogues, DEET, *E. citriodora* oil and aqueous fraction of the hydro-distillate.

Two phytochemical blends were assayed in the study, *E. citriodora* oil and water extract of the hydro-distillate. *E. citriodora* oil

Test Compound	% Repellency (± SE)				
	0.0001	0.001	0.01	0.1	1
(±) Cis-PMD	70.5 ± 3.9^{a}	77.1 ± 5.2^{a}	81.5 ± 5.8^{a}	86.2 ± 5.2^{a}	95.8 ± 3.9^{a}
(±) trans-PMD	70.7 ± 4.0^{a}	76.7 ± 5.7^{a}	80.7 ± 6.0^{a}	93.2 ± 4.8^{a}	95.8 ± 3.9^{a}
(−)-Trans-PMD	73.0 ± 5.7^{a}	75.3 ± 5.3^{a}	83.4 ± 5.8^{a}	85.1 ± 5.7^{a}	96.6 ± 3.7^{a}
(+)- Trans-PMD	73.5 ± 5.7^{a}	75.9 ± 5.1^{a}	79.0 ± 5.1^{a}	82.8 ± 5.3^{ab}	95.5 ± 4.1^{a}
DEET	73.4 ± 5.6^{a}	76.9 ± 5.6^{a}	83.1 ± 5.8^{a}	87.8 ± 5.1^{a}	97.0 ± 3.3^{a}

Table 2: % Repellency (±SE) of PMD stereoisomers and DEET at different doses (mg).

Means in columns followed by the same letters are not significantly different (P ≤ 0.05; SNK test).

Test Compound	Mean % Repellency (± SE) at different doses (g)				
	0.0001	0.001	0.01	0.1	1
L-Menthol	62.3 ± 4.2^{b}	75.5 ± 7.7^{b}	79.5 ± 7.4^{b}	91.2 ± 5.5^{b}	95.6 ± 4.1^{b}
1-α-Terpineol	33.8 ± 8.3^{c}	60.7 ± 7.5^{c}	77.8 ± 7.8^{b}	89.2 ± 6.1^{b}	96.5 ± 3.8^{b}
E.citriodora oil	81.1 ± 7.9^{a}	89.9 ± 6.4^{a}	98.9 ± 2.2^{a}	100 ± 0^{a}	100 ± 0^{a}
Aqueous Fraction	41.5 ± 2.9^{c}	57.0 ± 2.5^{c}	62.0 ± 4.8^{c}	66.3 ± 4.4^{c}	70.4 ± 5.2^{c}

Table 3: % Repellency (± SE) of PMD analogues and *E. citriodora* oil (g).

Means in columns followed by the same letters are not significantly different (P ≤ 0.05, SNK test).

RD_{75} of *p*-menthane-3,8-diol stereoisomers, analogues, DEET and *E. citriodora* oil	
PMD Stereoisomers and Essential Oil	RD_{75} ($\times 10^{-3}$ mg)
(−)-*Trans*-PMD	2.03^{d}
(+)-*Trans*-PMD	2.04^{d}
(±)-*Trans*-PMD	1.21^{d}
(±)-*Cis*-PMD	1.42^{d}
DEET	1.43^{d}
E. citriodora oil	189.33^{c}
L-Menthol	1734.12^{b}
1-α-Terpineol	6960.34^{a}
Aqueous Fraction	9425.44^{a}

Table 4: Probit analysis of dose-response relationship (RD_{75}) of *p*-menthane-3,8-diol (PMD) stereoisomers, analogues, DEET and *E. citriodora* oil.

Means in columns followed by the same letters are not significantly different (P ≤ 0.05; SNK test).

had much lower repellency than PMD stereoisomers, but significantly higher repellency than L-menthol and 1-α-terpineol (Table 2 and 3). Racemic citronellal and isopulegol are present in relatively large amounts in the terpenoid fraction of *E. citriodora* oil and are easily convertible to menthane diol. The aqueous fraction of the hydro-distillate was least repellent to the Brown ear tick (Table 4). It was found to contain aromadendrene oxide, linalool, citronellic acid and ursolic acid, which have been shown to be weakly repellent to arthropods [21].

Discussion

Two interesting structural effects on repellence may be highlighted. First, racemates ((±) - (*cis)*-PMD) and (±) - (*trans)*-PMD (*trans)*-PMD were as repellent as (+)-*Trans*-PMD and (-) - *(trans)*-PMD [22] Thus, repellency was neither stereospecific nor stereoselective. This shows that the precise orientation of the hydroxyl groups (at C-3 and C-8) in menthane diol skeleton is not important in conferring repellency against *R. appendiculatus.* From the practical standpoint, these results mean that one can obtain synthetic or semi-synthetic products of optimal repellent action against *R. appendiculatus* without regard to the stereochemical form of the starting material or the diol product. Interestingly, a similar result was also obtained against *Anopheles gambie s.s.* [23]. This suggests some similarity in the chemoreceptors and odorant-binding proteins (OBPs) of mosquitoes and ticks. Secondly, analogues of the diol (L-menthol, 1-α-terpineol) showed much lower repellency against *R. appendiculatus* compared to *p*-menthane-3,8-diol stereoisomers. Menthol (3) has the menthane ring as well as a hydroxyl group at C-3, while 1-α-terpineol has a menthane ring and a hydroxyl group at C-8. Thus, the presence of a second hydroxyl group in menthane diol is important for repellency against the Brown ear tick. Moreover, the presence of a double bond, which changes the shape of the molecule, further reduces the repellence of 1-α-terpineol. Previously, these PMD analogues were found to be attractive to *Anopheles gambiae*, particularly at higher doses [23] This indicates that although there are common features in the odorant-binding proteins (OBPs) of mosquitoes and ticks, as reflected in their behavioural responses to PMD stereoisomers, there are also significant differences. Unlike mosquitoes, ticks lack antennae and they detect host cues using sensilla located on the tarsi of the front legs. Their olfactory reception neurons may be narrowly tuned to specific odors [24,25]. The presence of two hydroxyl groups in PMDs raises their polarity, which would lower their volatility. This may be of particular advantage in substantially extending the rate of their evaporation, thus enhancing the longevity of their performance. *E. citriodora* oil had much lower repellency than PMD stereoisomers, but significantly higher repellency than L-menthol and 1-α-terpineol. The results show potential for use of menthane-diol stereochemical blend prepared by cyclization of (±)-citronellal obtainable in

good quantities from citronella grass as efficacious tick repellent.

Conclusions and Recommendations

This study has found out that PMD stereoisomers and *E. citriodora* oil possess potential repellency activity against the Brown Ear tick (*Rhipicephalus appendiculatus*). The study recommends use of stereosisomers of Menthane-diol and *E. citriodora* oil/ plant leaves by farmers in management of the Brown Ear tick (*Rhipicephalus appendiculatus*). Additionally, livestock farmers can grow citronella grass which can be used to synthesize PMD stereochemical via cyclization of (±) - citronellal.

Suggestion for Further studies

Further studies should be carried out to determine the mechanism of repellency from the extract. Other analogues of Menthanediol should be analyzed for brown tick repellency and other ectoparasites in livestock.

Conflict of Interests

The authors declare that they have no competing conflict of interests that could affect or influence publication of this paper.

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Authors Information

All authors have accepted to send the manuscript for publication to this journal, and declare no competing financial interest.

Disclosure

This article is part of Alex M. Muthengi Thesis [25], and no preprint version had been deposited in a journal in any form.

Data Availability

The data used to support the findings of this study are provided in this article. However, any additional information can be provided by the corresponding author upon request.

Ethical Approval

All the reagents used in this study were prepared, used, and disposed of according to the set laboratory guidelines and the material safety and data sheets (MSDS).

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