Phenolic and Monoterpenoid Mosquito Repellent Constituents of Headspace Vapors of *Conyza newii*

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Abstract

The plant Conyza newii has been reported to posses, mosquito repellent and fumigant toxicity properties. In this study headspace vapors emitted by the plant were obtained by adsorption on porapak Q® through headspace trapping under field conditions in West Pokot (35°E, 1°N) and Kericho (35°E, 0°) and analyzed using Gas Chromatography-Mass Spectrometry and co-injection with authentic standards. Mosquito repellency bioassays of volatiles and their synthetic blend were carried out according to the World Health Organization protocol for evaluation of repellents. Five major compounds identified in the volatiles were trans-limonene oxide, cis-limonene oxide, cis-dihyrocarvone, 4-methoxyphenol and 3-ethoxy-2-methyl phenol (2:2:2:2:1). Authentic standards of the compounds were bioassayed against Anopheles gambiae s.s and the three of them, trans-limonene oxide, cis-dihyrocarvone and 4-methoxyphenol exhibited repellency of $RD_{50} = 9.2 \times 10^{-5}$ mg cm,$^{-2}$, 95% CL, $RD_{50} = 5.4 \times 10^{-4}$ mg cm,$^{-2}$, 95% CL and $RD_{50} = 6.7 \times 10^{-4}$ mg cm,$^{-2}$, 95% CL respectively.Synthetic blend of the three compounds in their natural ratio (1:1:1) exhibited higher repellency (6.2×10^{-5} mg cm^{-2}) than the individual compounds and even the oil of the plant obtained through steam distillation.

Key words: *Conyza newii* Headspace trapping Volatiles Phenolic Monoterpenoid Mosquito Repellency

Introduction

Headspace trapping coupled to Solid Phase Microextraction (SPME) has been exploited previously to obtain plant volatiles from plants in their natural habitats[1]. The disadvantage of invasive extraction techniques such as hydro-distillation and solvent extraction is the potential change in volatile composition owing to plant stress and change in the physical environment of the plant. As a result, volatiles analyzed from detached plant tissues may be insignificant in determining the actual chemical profile of volatiles emitted by a particular plant in a natural ecological niche. Besides, the common high temperature distillation causes reduction in yield of key triterpenoids associated with plant essential oils[2,3]. Knowledge of chemical composition of volatiles emitted by plants in their natural habitats can contribute immensely to understanding plant-pathogen interaction and plant-plant communication[1]. In a previous communications, a much higher fumigant toxicity (2.0×10^{-3} mg cm^{-3}) and repellency ($RD_{50} = 8.9 \times 10^{-5}$ mg cm,$^{-2}$, 95% CL) of *Conyza newii* essential oil against the malaria vector *Anopheles gambiae s.s* has been reported[4,5,6]. The major constituents of the oil are mainly monoterpenoids including (*S*)-(-)-perillyl alcohol, (*S*)-(-)-perillaldehyde, geraniol, (*R*)-(+)-limonene. *trans-β*-ocimene and 1,8-cineol. In its natural habitat the plant posses a strong smell and is rarely infested by plant eating insects. There are no literature reports on the chemical composition of headspace vapors obtained from *C. newii* obtained by non-invasive methods of extractions. The present study reports the extraction and Gas Chromatography-Mass spectrometry analysis of headspace vapors of *C. newii* and mosquito repellency of a synthetic blend consisting of the constituents combined in their natural ration in the vapors.

Materials and methods

Headspace trapping

A wire mesh was folded to make adsorbent satchets measuring 2.5 × 4.0 cm. Porapak S (200 mg) was introduced into each satchet and sealed with staples. The filled satchets were cleaned in a sohxlet using HPLC grade dichloromethane for 4 days. The cleaned satchets containing the adsorbent were activated under nitrogen in a purpose-adapted gas chromatograph oven. A tripod stand was used to hold an inverted funnel and a suspended satchet of adsorbent material just above the leaves of the plant. The plant was completely covered by the funnel to minimize loss of volatiles. A control experiment was set up in the same way at the same site but with no *C. newii* plant underneath. The experiment was stopped after 12 hours and repeated for several plants to collect enough material for analysis. The test and control satchets were eluted separately with 2 ml of HPLC grade dichloromethane and the eluate collected. Both eluants were kept at -4^{o}C on ice until required for analysis.

Gas-Chromatography-Mass Spectrometry analysis.

GC-MS analysis of the volatiles trapped was carried out as described by Omollo[7]. Separation of oil constituents was done on a Hewlett Packard (HP) 5890 series II capillary GC equipped with split less capillary injection system and a flame ionization detector coupled with an integrator (HP 3393 A series II). Resolution was done on 50 m × 2 mm (i.d) × 0.33 µm (film thickness) cross-linked methylsilicone capillary column. White spot nitrogen was used as a carrier gas at flow rate of 0.7 ml/min. The temperature program was 50 (5 min) – 280 (10 min) at 5^{o}C/min. GC-MS analysis was carried out on a HP 8060 series II GC linked to VG platform MS. The temperature of the source was set at 180^{o}C and a multiplier voltage of 300 V.

Mosquito Repellency Bioassay

The mosquito repellent activity of individual compounds identified in the vapors and a blend of the compounds in their natural ratios was established according to World Health Organization protocols for laboratory and field evaluation of insecticides and repellents as described by Omollo[7].

Bioassays were carried out in a dark room with red light as the only source of illumination. The room temperature and humidity was artificially set to mimic the feeding conditions of female *An. gambiae s.s* (27-35oC, RH ≥ 65%). All repellency tests were carried with on 5-7 day old *An. gambiae s.s* mosquitoes. Six human volunteers were used in repellency bioassays using 0.01, 0.1, 1.0 and 10% solutions of essential oil in acetone. A total of 18 cages each measuring 50 × 50 cm were used with each containing 25 starved female *An. gambiae s.s* mosquitoes. Test solution (10 ml) was dispensed on one forearm of a volunteer from the elbow to the wrist. The rest of the arm was covered with a glove to make it unattractive to mosquitoes. Acetone (10 ml) was dispensed on the other arm in the same way to act as a control. The control arm was placed into the cage containing test mosquitoes immediately after releasing 25 insects and kept there for 3 minutes. The number of mosquitoes landing on both control and treated arms were recorded separately. The screening was done sequentially beginning with the lowest dose (control) to the highest (10%). Each concentration was screened with a fresh batch of mosquitoes. After bioassay of each concentration, the arms were washed with bar soap, rinsed well with tap water and dried using a clean towel for 15-20 minutes before application of next dose sample.

Data Analysis. The % protective efficacy (PE) was calculated as follows

PE = (PCM-PTM)/PCM X 100

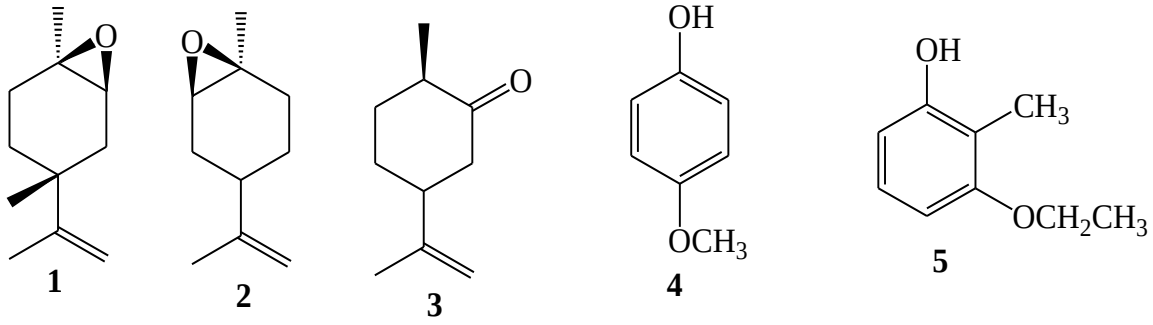
Where PCM and PTM is the % control and treated means, respectively[8].

Results and discussion

Chemical composition of *C. newii* headspace vapors

The major compounds identified in the vapors of the plant growing in the two

regions were *cis*-limonene oxide (**1**), *trans*-limonene oxide (**2**), *cis*-dihydrocarvone, 4-methoxyphenol (**3**) and 3-ethoxy-2-methylphenol (**4**) (Table 1).



1 **2** **3** **4** **5**

Table 1: The chemical composition of vapors obtained from *Conyza newii* leaves by headspace trapping

	Relative abundance (%)	
Compound	Nyakach	Kericho
cis-limonene oxide	18	20
trans-limonene	12	14
cis-dihydrocarvone	41	41
4-methoxyphenol	28	24
3-methoxy-2-methylphenol	t	t

The relative concentration of *cis*-dihydrocarvove and 4-methoxyphenol were high in the two regions. *Cis* and *trans*-limonene oxide occur in low amounts while 3-methoxy-2-methylphenol occurs in trace amounts.

Mosquito repellent activity of synthetic standards of compounds identified in the vapors

Due to very low amounts of headspace trapping volatiles, standards of three compounds (*trans*-limonene oxide, *cis*-dihydrocarvone and 4-methylphenol) obtained by headspace trapping were bioassayed (Table 2). These compounds exhibit appreciable repellent activity but none achieved 100% protective efficacy even at 10% concentration in acetone.

Table 2: Mosquito repellent activity of some compounds identified in headspace vapors of *C. newii*.

	Mean % protective efficacy		
Concentration	trans-limonene oxide	cis-Dihydrocarvone	4-methoxyphenol
0.01	28.61 ± 1.41	21.03 ± 2.08	14.4 ± 1.81
0.1	41.59 ± 2.21	30.44 ± 1.41	23.15 ± 2.11
1	63.85 ±2.18	53.21 ± 2.21	31.32 ± 1.29
10	85.40 ± 1.71	62.88 ± 1.71	53.83 ± 1.41

A mixture containing trans-limonene oxide, *cis*-dihydrocarvone and 4-methoxyphenol in their natural ratios (1:1:1) was prepared and 0.01% concentration of the blend bioassayed against *Anopheles gambiae s.s* (Table 3).

Table 3: mosquito repellent activity of a blend of three major compounds identified in *C. newii* headspace vapors.

Concentration	Mean % Protective efficacy
0.01	32.58 ± 0.42
0.1	54.09 ± 0.21
1	67.9 ± 0.34
10	100

Whereas no individual compound achieved 100% repellency, the blend containing the compounds present in headspace vapors achieve 100% repellency at 10%. From the results of the present study, the compounds reported in the headspace vapors are markedly different from those reported in the essential oil of the plant. Note that apart from *cis*- and *trans*-limonene oxide and dihydrocarvone which are monoterpenoids in the same chemical family to those present in the essential oil obtained by hydro-distillation, headspace vapors also contain aromatic compounds (4-methoxyphenol and 3-methoxy-2-methylphenol).

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