SHORT COMMUNICATION

Repellency property of long chain aliphatic methyl ketones against *Anopheles gambiae* s.s

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Abstract: Long chain aliphatic methyl ketone series of C_7-C_{15} were tested for repellency activity against the malaria transmitting mosquito *Anopheles gambiae* s.s. All methyl ketones produced a dose dependent (P<0.001) repellency response with 2-tridecanone giving comparable protection efficacy to DEET at 10% and 1% concentrations. Aliphatic methyl ketones of C_7-C_{10} had lower activity than those of C_{11}-$C_{15.}$ However, within this range compounds with odd carbon atoms (2-undecanone, 2-tridecanone and 2-pentadecanone) were more effective than compounds with even carbon atoms (2-decanone and 2- dodecanone). Comparable repellency activity of 2-tridecanone to DEET show that, it may save as *Anopheles gambiae* s.s. mosquito repellent.

Keywords: Long chain aliphatic methyl ketones; Insect repellents; *Anopheles gambiae* s.s.

Repellency Repellent properties of some plants to mosquitoes are well-known. Phytochemicals obtained from huge diversity of plant species are an important source of safe and biodegradable chemicals, which could be screened for mosquito repellent activities (http://www.mrcindia.org/MRC profile/alternate strategy/plant ins.pdf).Mosquito repellents have a unique role in malaria endemic regions, especially in the evening before bedtime. Besides protection against the nuisance biting, they reduce human vector contact hence minimize incidences of malaria transmission (Xue et al, 2001). N,N-diethyl-m-toluamide (DEET), a synthetic repellent, has been effective in repelling various insects including mosquitoes (WHO, 2003). However, DEET has been reported to be an irritant substance when applied to the skin and it causes central nervous system disturbances when in prolonged use (Lewis, 1996; Nkunya, 2000), hence the need to search for more alternative mosquito repellents.

In Africa, several plant species are known to reduce mosquito biting activity when used as repellents.These include *Hyptis suaveolens* Poit. (Lamiaceae), Daniellia oliveri Rolfe (Caesalpiniaceae), *Elaeis guineensis* Jacq. (Arecaceae), smoke of the seed capsules of *Parkia biglobosa* (Jacq.) Benth. (Mimosaceae), Azadirachta indica A.Juss. (Meliaceae), *Eucalyptus* sp. (Myrtaceae), *Ocimum canum* Sims (Lamiaceae), and fresh *Senna occidentalis* (L.) Link (Caesalpiniaceae) (Pålsson & Jaenson, 1998).

Previous studies of long chain aliphatic methyl ketones showed repellence to arthropods, including blood sucking insects (Ndungu *et al.*, 1995; Blum, 1966; Torr *et al.,* 1996; Barton, 2003; Roe, 2004; Gikonyo *et al.,* 2002;). For example, repellency activities of 2-decanone, 2-undecanone and 2-dodecanone to *Glossina morsitans morsitans* have been recorded in wind tunnel experiments (Gikonyo *et al*, 2003). 2-octanone reduces the catch of baited trap of *G. morsitans morsitans* and *G. pallidipes* in the field (Torr *et al.,* 1996). 2-heptanone is both an alarm pheromone as well as a defensive allomone released by the dolichoderine ant *Iridomyrnex pruinosus* (Blum *et al.,* 1966; Brown, 1980), beetle in the genus *Dyschirius* (Moore & Brown, 1979) and Honeybee in the genus *Apis* (Shearer & Boch, 1965; Brown, 1980; Malerbo- Souza & Nogueira-Couto, 2004). 2-Dodecanone which was isolated from essential oil of *Cleome monophyla* (Capparaceae) was repellent to brown ear tick, *Rhipicephalus appendiculatus* (Ndungu *et al*, 1995). 2-undecanone and 2-tridecanone, which are found in the gradular trichomes of wild tomatoes of the genus *Lycopersicon* (Solanaceae) are repellent to *Culex quinquefasciatus* and *Aedes aegypti* mosquitoes (Barton, 2003; Roe, 2004). 2-undecanone applied at 30% and 40% solution (vol/vol) in isopropyl alcohol to human skin was 100% active for 15 min. in preventing *Ae. aegypti* mosquito feeding on human skin (Barton, 2003). In another study, repellent activity for 2-undecanone at 100% and 50% concentration, showed residual activity of 100% at 6 hours when tested against *Cx quinquefasciatus* (Roe, 2004). This activity was more effective than 20% DEET for the comparable periods (Roe, 2004). 2-tridecanone at 100% and 50% concentration showed strong repellent activity against *Cx quinquefasciatus*

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immediately after treatment and there was some residual activity after 6 hours (Roe, 2004). Therefore, since some insect cues are species specific (Collin *et al*, 1993; Faradin & Day, 2002) and due to the above mentioned repellency evidences of long chain aliphatic methyl ketones to various insects, follow up studies were conceived, in order to assess their potency as *Anopheles gambiae* s.s. repellent candidates.

Female adult *Anopheles gambiae* s.s. mosquitoes (Ifakara strain) were used in all experiments as supplied by the International Centre of Insect Physiology and Ecology (ICIPE) insectary unit in Nairobi, Kenya. The mosquito colony was originally obtained from Ifakara in Tanzania in 1996 and reared under standard insectary conditions at ICIPE. The larvae were reared in the insectary room where temperature was maintained at 32 - 36oC and fed on TetraMin® food (Tetra GmbH, Germany). Adult mosquitoes were fed on a 6% glucose solution and breeding females fed on human blood thrice a week. Rearing temperatures and relative humidity in the adult insectary were maintained at 26 - 28 oC and 70 - 80 %, respectively. Mosquitoes used in the experiment were 5 - 7 days old, initially fed on glucose (6 % solution) and then starved for 18 h, before carrying out the assays.

Authentic samples of 2-methyl ketones and N,N-diethyl-m-toluamide were used for this investigation (Table 1). The repellency assays were performed in a dark room with red light as the only source of illumination (WHO, 1996). The room temperature and humidity were controlled at 28 ± 2 oC and 70 ± 5 %, respectively to mimic the feeding conditions of female *An. gambiae* s.s. mosquitoes. Cages (50cm x 50cm x 50cm) made with aluminium sheet at the bottom, window screen made of pyrex on sides and top, and a cotton sockinet sleeve for accessing on the front, were used to study the dose responses.

Different concentrations of samples (0.01% – 10%) were prepared by dissolving 1.0g of the samples in 10 ml of acetone followed by ten-fold dilution to obtain the subsequent concentrations. Acetone acted as a blank in all experiments. Fifty test mosquitoes and five adult human volunteers (3 males and 2 females) who did not apply any lotion, perfume, oil or perfumed soap on the day of the bioassay were used. The forearm (average area of 696.6 cm2) of each volunteer from the elbow was washed with water, left to dry and then introduced with the hand covered by a glove in order to be unattractive to mosquitoes. The test sample (1 ml) was spread evenly over the treatment area and presented one after the other to the same caged mosquitoes for a particular sample and for a particular person. Sequential exposure to high dosages (0.01, 0.1, 1.0 and 10 %) of repellent for 3 min (one) was done. The right arm was used for the treatment while the left arm was used as a control. After bioassay of each concentration, the hands were washed using a non-perfumed soap and tap water and allowed to dry naturally for a period of about 20 min. before dispensing the subsequent concentration. The number landing on or probing the arm was counted and recorded for each volunteer. Mosquitoes were shaken off the arm before they imbibed any blood. Exposure of treatment arm and control arm was alternated to provide a standard for comparing avidity of biting

Percentage protective efficacy (PE) was calculated using the formula PE = (C-T/T) x100%, where C and T are the mean numbers of mosquitoes that landed on the control and test hand, respectively (Sharm & Ansari, 1994; Matsuda *et al,* 1997; Yap *et al,* 1998). Data were subjected to one way analysis of variance (ANOVA) and mean percentage protection were compared using Student-Newman-Kuels (SNK) of the SAS package (SAS, 2000).

All methyl ketones tested in this study exhibited good protection efficacy against *An. gambiae* s.s, except for 2-heptanone and 2-octanone (Table 2) ($P<0.001$). These activities were dose-dependant. 2-dodecanone and 2-tridecanone gave 100% protection efficacy at 10% concentration, although volatility of compounds seemed to inhibit further activity at low concentration levels. 2-tridecanone gave comparable protection efficacy to DEET except at 0.1% concentration. Aliphatic methyl ketones of C7-C10 had lower activity that those of C11-C15 showing the significance of chain length to the observed activities. However, in the C11-C15 aliphatic methyl ketone series, compounds with odd carbon atoms (2-undecanone, 2-tridecanone and 2-pentadecanone) were more effective than compounds with even carbon atoms (2-decanone and 2-dodecanone) especially at high concentration levels. Due to the observed trend, it was concluded that, repellency activity was the result of differential ability of these compounds to penetrate to the active site of the *Anopheles gambiae* s.s. sensilla. Comparable repellency activity of 2-tridecanone to DEET shows that, formulations may optimize its performance as *An. gambiae* s.s. repellent candidates to be used for reducing human-vector contact thereby minimizing incidence of malaria. The fact that long chain aliphatic methyl ketones repels arthropods, including blood sucking insects calls for more investigations of the methyl ketones analogies as potential source of insect repellent including mosquitoes.

Table 1: Chemical compounds which were tested against *An. gambiae s.s*

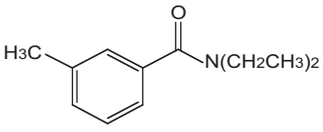
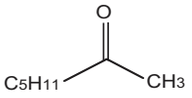
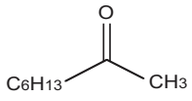
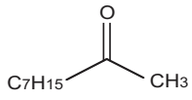
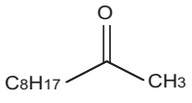
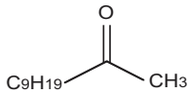
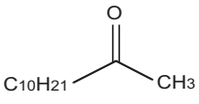
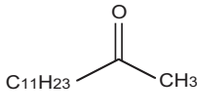
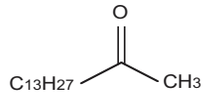
Compound name	Chemical molecular formula	Purity/ grade (%)	Manufacturer/ Country
DEET		97	Rieldel-De Haen in U.K
2-Heptanone		GC grade	Aldrich Chemical Company, Inc. USA
2-Octanone		99	Aldrich Chemical Co Ltd Gillingham, Dorset England
2-Nonanone		99	Aldrich Chemical Co Ltd Gillingham, Dorset England
2-Decanone		95	Aldrich Chemical Co Ltd Gillingham, Dorset England
2-Undecanone		99	Sigma-Aldrich Chemie GmbH, German
2-Dodecanone		97	Fluka chemika no 30720 Switzerland
2-Tridecanone		99	Aldrich Chemical Co Ltd Gillingham, Dorset England
2-Pentadecanone		99	Aldrich Chemical Co Ltd Gillingham, Dorset England

Table 2: Protection efficacy of long chain methyl ketones (± SE) to *An. gambiae* s.s bite

Compound name	Concentration (% w/v)			
	0.01	0.1	1	10
2-Heptanone	12.3 ± 3.1^{bB}	17.8 ± 0.9^{dB}	26.8 ± 4.3^{dAB}	38.2 ± 7.5^{cA}
2-Octanone	16.8 ± 3.0^{bC}	30.6 ± 3.1^{cB}	32.9 ± 4.6^{bcB}	69.0 ± 6.6^{bA}
2-Nonanone	37.2 ± 4.2^{aC}	42.6 ± 3.6^{bBC}	53.3 ± 4.2^{bcB}	86.2 ± 3.6^{aA}
2-Decanone	8.0 ± 2.7^{bC}	13.8 ± 2.1^{dC}	34.0 ± 5.7^{bcB}	83.4 ± 4.5^{aA}
2-Undecanone	35.2 ± 6.3^{aCD}	50.9 ± 4.6^{bBC}	64.4 ± 7.7^{bB}	91.8 ± 5.0^{aA}
2-Dodecanone	24.3 ± 6.2^{abC}	27.1 ± 4.1^{bC}	53.9 ± 9.3^{bcB}	100 ± 0^{aA}
2-Tridecanone	23.5 ± 4.3^{bD}	44.2 ± 2.1^{bC}	88.3 ± 7.3^{aB}	100 ± 0^{aA}
2-Pentadecanone	17.6 ± 2.9^{bD}	41.6 ± 3.6^{bC}	65.6 ± 6.1^{bB}	93.3 ± 4.2^{aA}
DEET	13.1 ± 2.6^{bC}	82.7 ± 1.9^{aB}	100 ± 0^{aA}	100 ± 0^{aA}

Mean values with the same small letters within the same concentration level and mean values with the same capital letters for a particular treatment are not significantly different at p>0.001 by student-Newman-Kuels (SNK)

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