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Insecticidal activity of menthol derivatives against mosquitoes†

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Abstract

BACKGROUND: The insecticidal activity of essential oil of *Mentha piperita* L. emend. Huds. against local mosquitoes as disease vectors was recognized and found to be due to the presence of menthol, which is the major aroma compound of the oil. The minor compounds of the oil, i.e. menthone, β-caryophyllene, menthyl acetate, limonene, α-pinene and pulegone, showed either less or no activity against the mosquitoes tested. L-Menthol derivatives were synthesized and their knockdown effect and mortality were evaluated against local mosquitoes of *Culex quinquefasciatus* Say, *Aedes aegypti* L. and *Anopheles tessellatus* Theobald as disease vectors. This is the first report of mosquitocidal activity of menthol and its derivatives against *Cx. quinquefasciatus, Ae. aegypti* and *An. tessellatus*.

RESULTS: Derivative synthesis followed by structure–activity relationship studies identified several derivatives, i.e. menthyl chloroacetate, menthyl dichloroacetate, menthyl cinnamate, menthone glyceryl acetal, thymol, α-terpineol and mugetanol, with enhanced mosquitocidal activity against *Cx. quinquefasciatus, Ae. aegypti* and *An. tessellatus* relative to the parent compound L-menthone.

CONCLUSION: In ester derivatives of L-menthol the optimum activity is dependent on the size and shape of the ester group and the presence of chlorine atoms in the ester group. In structurally related derivatives of L-menthol the optimum activity is dependent on the aromaticity, the degree of unsaturation, the position of the hydroxy group and the type of functional group.

Keywords: menthol; insecticidal activity; mosquitoes; *Culex quinquefasciatus*; *Aedes aegypti*; *Anopheles tessellatus*; structure-activity relationships

1 INTRODUCTION

Public health pest control is a major priority to minimize infection by and transmission of vector-borne diseases in tropical regions. Diseases transmitted by mosquitoes, such as malaria, lymphatic filariasis and numerous viral diseases such as dengue, dengue haemorrhagic fever, Japanese encephalitis and yellow fever, affect billions of people worldwide including Sri Lanka each year. The development of resistance by vector mosquitoes in Sri Lanka[1,2] is reducing the effectiveness of synthetic insecticides such as pyrethroids in mosquito control. Also, many of the synthetic insecticides, especially chlorinated hydrocarbons, are toxic to humans and have detrimental environment effects. The adverse effects of synthetic insecticides have necessitated the search for more acceptable plant-based products for mosquito control. In recent years, plant essential oils and essential oil constituents have been considered as potent alternatives to conventional insecticides as a natural means of pest control.[3,4] Insect control properties of plant essential oils and their constituents and derivatives have been described previously.[5–10] However, there have been no previous publications on the mosquitocidal activity of menthol and its derivatives.

In search of insecticidal properties of plant essential oils, the insecticidal activity of *Mentha piperita* L. emend. Huds. oil was recognized against local mosquito disease vectors and shown to be due to the presence of menthol, which is the major aroma compound of the oil. Previous publications on the insecticidal activity of menthol and menthol derivatives cover only the compounds menthyl acetate and pivalate, which were described as insecticidal against *Musca domestica* L.[5,11] The other reported biological activities of menthol and its derivatives include

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antimicrobial,[12,13] anti-inflammatory,[13] analgesic[13,14] and central nervous system excitation effects,[13] and insecticidal activity against stored-product pests.[15] Menthol and menthol derivatives are commonly used as cooling agents in cosmetic products and also as flavouring for toothpaste, other oral hygiene products and chewing gum.[14]

In the present study, *M. piperita* oil, its major aroma compound, menthol, and synthesized menthol derivatives were investigated for mosquitocidal activity, and the activity of menthol derivatives was compared with that of L-menthol in order to evaluate the effect of derivatization and to establish structure–activity relationships (SARs) of the compounds with the aim of identifying structural features that are necessary for mosquitocidal activity.

2 EXPERIMENTAL METHODS

2.1 Essential oil, compounds and chemicals used

The compounds tested are shown in Figs 1 and 2. *Mentha piperita* (mint) oil was purchased from Hendrikson & Sons Pvt Ltd., Colombo, Sri Lanka. L-Menthol, L-menthone, thymol, α-terpineol, pulegone and mugetanol were purchased from Haarmann & Reimer GmbH, Holzminden, Germany. Chemicals and reagents used for derivative synthesis were purchased from Fluka Chemie GmbH, Buchs, Switzerland, and VWR International Ltd, Poole, UK, and were analytical grade.

2.2 Derivative synthesis

Acyl and aryl derivatives of L-menthol, i.e. menthyl acetate, chloroacetate, dichloroacetate, trifluroacetate, propionate, chloropropionate, pivalate and benzoate, were synthesized by reaction with acid anhydrides or acid halides in the presence of a catalytic quantity of pyridine or triethylamine in dichloromethane at room temperature.[7,16–18] Acyl derivatives of L-menthol with an acid moiety (lactic acid or cinnamic acid), i.e. menthyl lactate and cinnamate, were synthesized by reaction with dicyclohexylcarbodiimide and 4-dimethylaminopyridine as condenser and catalyst in dichloromethane at room temperature.[7] The methoxy derivative of L-menthol, menthyl methyl ether, was synthesized by reaction with dimethyl sulfate and anhydrous potassium carbonate under reflux in dry acetone.[19] The glyceryl acetal derivative ofL-menthol was synthesized by reaction with glycerol and *p*-toluenesulfonic acid under reflux in toluene.[20] Reactions were monitored by thin-layer chromatography, using a mixture of hexane and ethyl acetate, and compounds were visualized by vanillin-sulfuric acid spray, heat and under UV light. Compounds were purified by dry-column flash chromatography,[21] eluting with hexane + ethyl acetate.

2.3 Gas chromatography (GC) analysis

Constitutional analysis of *M. piperita* oil was carried out on a gas–liquid chromatograph (Shimadzu GC-2010, Shimadzu, Japan) equipped with flame

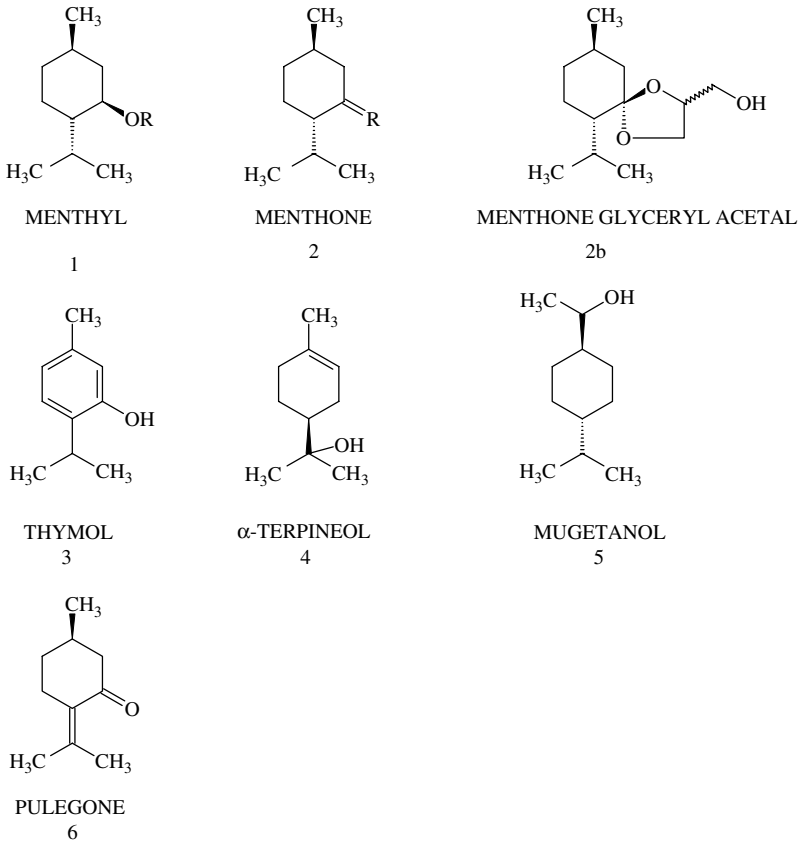


Figure 1. Structures of compounds discussed (for R, see Fig. 2).

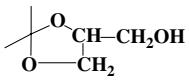
	DERIVATIVE[a]	R[b]
1a	Menthol (parent)	—H
1b	Acetate	—C(=O)—CH_3
1c	Chloroacetate	—C(=O)—CH_2Cl
1d	Dichloroacetate	—C(=O)—$CHCl_2$
1e	Trifluoroacetate	—C(=O)—CF_3
1f	Propionate	—C(=O)—CH_2CH_3
1g	Chloropropionate	—C(=O)—CH_2CH_2Cl
1h	Pivalate	—C(=O)—$C(CH_3)_3$
1i	Benzoate	—Bz
1j	Lactate	—C(=O)—$CH(OH)CH_3$
1k	Cinnamate	—C(=O)—CH=CH—Ph
1l	Methyl ether	—CH_3
2a	Menthone (parent)	=O
2b	Glyceryl acetal	

Figure 2. Menthol and menthone derivatives synthesized ([a]compound identification name and number in Table 1; [b]R in Fig. 1).

ionization detection. An SGE BP-20 capillary column 30 m × 0.25 mm, film thickness 0.25 µm, was used. Oven temperature was programmed from 60 to 225 °C at 5 °C min^{-1}. Injector and detector temperatures were kept at 230 and 240 °C respectively. Argon was used as the carrier gas at a flowrate of 1.00 mL min^{-1}. The compounds of *M. piperita* oil were identified by comparing the GC retention times with those of authentic standard compounds and by the peak enrichment technique.

2.4 Spectroscopy

The chemical structures of the menthol compounds synthesized, menthyl acetate, chloroacetate, dichloroacetate, trifluoroacetate, propionate, chloropropionate, pivalate, benzoate, lactate and cinnamate, menthyl methyl ether and a racemic mixture of menthone glyceryl acetal, were confirmed by ^{1}H and ^{13}C NMR spectral data.[22,23] ^{1}H and ^{13}C NMR were recorded on Varian Magnetic 300 MHz and Varian Gemini 200 MHz spectrometers (Varian, Inc., USA) using deuterochloroform as solvent.

2.5 Insecticidal assay

2.5.1 *Mosquitocidal bioassay*

Menthol compounds and *M. piperita* essential oil were tested for knockdown effect and mortality against three-day-old adult females of *Culex quinquefasciatus* Say, *Aedes aegypti* L. and *Anopheles tessellatus* Theobald. The mosquitoes tested are all Sri Lankan strains and have been colonized in the insectary for 10 years at the Medical Research Institute, Sri Lanka. Mosquitoes were reared under laboratory conditions in the insectary at 27 °C and 80% relative humidity and under a 14:10 h light:dark photoperiod.

The bioassay was conducted in an experimental kit consisting of two cylindrical plastic tubes both measuring 125 × 44 mm following the WHO standard method for adult mosquito susceptibility testing[24] using papers impregnated with 1.5 mL of 20, 10, 5, 2.5 or 1.25 g L^{-1} ethanol solutions of the compounds/essential oil. One tube served to expose the mosquitoes to the compounds/essential oil, and another tube was used to hold the mosquitoes before and after the exposure periods. The impregnated papers were rolled and placed in the exposure tube. Fifteen glucose-fed adult female mosquitoes were exposed to the impregnated papers, and the number of mosquitoes knocked down after a 1 h exposure period was recorded. Mosquitoes were transferred to a holding tube and kept for 24 h under laboratory conditions. A cotton pad soaked in 20% glucose solution was placed in the tube during the holding period of 24 h. Mortality of the mosquitoes was recorded after 24 h, and results were expressed in terms of mass of test compound per unit volume of the test apparatus (µg mL^{-1}). An ethanol control was carried out simultaneously with the test, and knockdown and mortality were zero. Each test was carried out in triplicate.

2.5.2 *Data analysis*

KD_{50} and LC_{50} values and 95% fiducial limits were calculated from the values obtained for knockdown and mortality with 20, 10, 5, 3, 2.5 and 1.25 g L^{-1} solutions of the compounds/essential oil. Data were analysed by probit analysis using an SPSS statistical package.[25,26]

3 RESULTS AND DISCUSSION

Although the insecticidal properties of certain essential oils, their constituents and derivatives have been well established,[4–8,11,15,16,27] studies on the activity of these against adult mosquitoes have not been previously reported except in the authors' ongoing work.[9,10,28] In preliminary studies, *M. piperita* essential oil showed good mosquitocidal activity, and hence the oil was analysed for its chemical composition by GC and the insecticidal activity of the major components was tested against local disease vector mosquitoes. GC analysis of the leaf oil of *M. piperita* showed that menthol (41.2%) was the major constituent and

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menthone (24.3%), β-caryophyllene (5.1%), menthyl acetate (2.0%), limonene (1.1%) and α-pinene (1.1%) were the other constituents present in >1% of the oil. It was found that pulegone was present in the oil in 0.07%.

Menthol, a major constituent of *M. piperita* leaf oil, showed more mosquitocidal activity against *An. tessellatus* (LC_{50} 0.36 and KD_{50} 0.54 μg mL^{-1}) and *Cx. quinquefasciatus* (LC_{50} and KD_{50} 0.50 μg mL^{-1}) than minor constituents of the oil, i.e. menthone, β-caryophyllene, menthyl acetate and pulegone (Table 1). However, *M. piperita* oil, menthol, menthone, β-caryophyllene, menthyl acetate and pulegone showed either less or no activity against *Ae. aegypti* (Table 1).

Synthesis of derivatives of L-menthol resulted in 12 compounds, menthyl acetate, chloroacetate, dichloroacetate, trifluoroacetate, propionate, chloropropionate, pivalate, benzoate, lactate and cinnamate, menthyl methyl ether and a racemic mixture of menthone glyceryl acetal, in good yield (Fig. 2). The structures of the above compounds were characterized by their ^{1}H and ^{13}C NMR spectral data and by comparison with the reported data of the parent compound.[22,23]

The 12 menthol derivatives synthesized (Fig. 2) and two standard compounds, menthyl anthranilate and menthyl carboxamide, were tested for knockdown effect and mortality against *An. tessellatus, Cx. quinquefasciatus* and *Ae. aegypti* following the WHO protocol.[24] Of the tested compounds, menthyl chloroacetate showed the highest mortality (LC_{50} 0.08 μg mL^{-1}) and menthone glyceryl acetal the highest knockdown effect (KD_{50} 0.19 μg mL^{-1}) against *An. tessellatus*. Menthyl benzoate, dichloroacetate, chloropropionate, carboxamide and lactate also showed good activity against *An. tessellatus* (Table 1). Menthyl chloroacetate showed the highest activity against both *Cx. quinquefasciatus* and *Ae. aegypti*, but the highest knockdown effect against *Cx. quinquefasciatus* was shown by menthyl dichloroacetate (Table 1). Menthyl trifluoroacetate, propionate, pivalate, benzoate and anthranilate were non-toxic at 20 g L^{-1} against *Cx. quinquefasciatus* and *Ae. aegypti*.

The mosquitocidal activity of L-menthol was compared with its structurally similar analogues, L-menthone, thymol, α-terpineol, pulegone and mugetanol. L-Menthol showed the highest mortality (LC_{50} 0.36 μg mL^{-1}) and mugetanol the highest knockdown effect (KD_{50} 0.31 μg mL^{-1}) against *An. tessellatus*. Mugetanol showed the highest knockdown effect against *Ae. aegypti* (KD_{50} 0.36 μg mL^{-1}) and α-terpineol the highest mortality (LC_{50} 0.62 μg mL^{-1}). Mugetanol showed the most activity against *Cx. quinquefasciatus* (KD_{50} and LC_{50} 0.17 μg mL^{-1}).

Menthol derivatives (Fig. 2) obtained by semi-synthetic modifications of the hydroxyl group of L-menthol showed enhanced mosquitocidal activity. Of these derivatives, menthyl chloroacetate, chloropropionate, dichloroacetate, carboxamide and menthone glyceryl acetal showed more mosquitocidal activity than menthol, menthone and menthyl methyl ether, indicating that the presence of ester groups, which make the molecule more lipophilic, enhances the mosquitocidal activity more than the hydroxyl, ether and carbonyl functionalities. The conversion of the carbonyl group in L-menthone by derivatization to an acetal group in menthone glyceryl acetal significantly enhanced the insecticidal activity, indicating that the presence of two oxygen atoms in a hemiacetal ring makes a significant contribution towards enhancing the activity. It was also observed that an alcohol, L-menthol, was more effective than the analogues ketone and L-menthone in the mosquitocidal bioassays.

It also appeared that derivatization of L-menthol with less bulky groups such as acetate tends to decrease the mosquitocidal activity below that of more bulky acyl and aryl groups, and this was supported by the derivatives, menthyl acetate and methyl ether, which showed either weak or no mosquitocidal activity, and by menthone glyceryl acetal, which showed more mosquitocidal activity. Among the ester derivatives, menthyl choroacetate, dichloroacetate and chloropropionate showed greater activity against the mosquitoes tested than acetates and propionates owing to the presence of the chlorine atom in the ester group. The trifluoroacetate derivative showed either weak or no activity against the tested mosquitoes at 20 g L^{-1}, indicating that the presence of more than one halogen atom tends to decrease activity. The presence of a nitrogen atom in the esterifying group retained the mosquitocidal activity against *An. tessellatus* and *Ae. aegypti*.

Structurally related analogues of menthol, i.e. thymol, α-terpineol, pulegone and mugetanol (Figs 1 and 2), have either comparable mosquitocidal activity with menthol or more mosquitocidal activity (Table 1), indicating that structural variations in the molecule could contribute either towards retaining or towards enhancing the activity. Thymol showed higher activity against *An. tessellatus* and *Ae. aegypti* than L-menthol, indicating that aromaticity is also a key factor in enhancing the activity of menthol. α-Terpineol showed more mosquitocidal activity against *Ae. aegypti* than L-menthol and comparable activity against *An. tessellatus* and *Cx. quinquefasciatus*, indicating that the double bond in the cyclohexane ring contributes to the mosquitocidal activity. The position of the hydroxyl functionality, i.e. either in the cyclohexane ring or in the branched chain of the molecule, may affect the mosquitocidal activity because mugetanol and α-terpineol both showed more mosquitocidal activity against *Cx. quinquefasciatus* and *Ae. aegypti*

Table 1. Insecticidal activity of menthol derivatives against mosquitoes

	Activity[a] (μg mL^{-1}) (95% FL)[b]					
	Anopheles tessellatus		*Culex quinquefasciatus*		*Aedes aegypti*	
Compound	KD_{50}	LC_{50}	KD_{50}	LC_{50}	KD_{50}	LC_{50}
1a Menthol	0.54 (0.46–1.04)	0.36 (0.24–0.45)	0.50 (0.41–0.62)	0.50 (0.32–0.76)	NA[c]	NA
1b Menthyl acetate	NA	NA	1.27 (1.13–1.44)	0.76 (0.07–1.35)	WA[d]	WA
1c Menthyl chloroacetate	0.57 (0.42–0.74)	0.08 (0.06–0.11)	0.40 (0.03–0.61)	0.26 (0.13–0.36)	0.62 (0.16–0.90)	0.75 (0.58–0.93)
1d Menthyl dichloroacetate	0.86 (0.69–1.03)	0.46 (0.19–0.63)	0.30 (0.04–0.72)	1.41 (1.10–1.99)	1.80 (1.49–2.57)	2.01 (1.69–3.05)
1e Menthyl trifluoroacetate	2.66 (2.24–5.15)	2.18 (1.70–3.79)	NA	NA	NA	NA
1f Menthyl propionate	2.11 (1.68–4.18)	1.82 (1.53–2.49)	NA	NA	NA	NA
1g Menthyl chloropropionate	0.34 (0.26–0.44)	0.28 (0.22–0.35)	2.02 (1.65–2.74)	2.36 (2.20–2.61)	NA	NA
1h Menthyl pivalate	3.26 (2.86–3.98)	NA	NA	NA	NA	NA
1i Menthyl benzoate	0.62 (0.34–0.86)	0.90 (0.51–1.43)	NA	NA	NA	NA
1j Menthyl lactate	0.59 (0.40–0.73)	0.80 (0.44–1.42)	1.55 (1.18–2.54)	2.32 (2.16–2.52)	NA	NA
1k Menthyl cinnamate	1.65 (1.31–2.25)	0.82 (0.59–1.09)	1.05 (0.80–1.36)	0.67 (0.48–0.86)	2.71 (2.24–3.66)	3.37 (2.59–6.06)
1l Menthyl methyl ether	NA	NA	NA	WA	NA	WA
Menthyl anthranilate[e]	NA	WA	NA	WA	NA	NA
Menthyl carboxamide[e]	2.23 (1.85–2.85)	1.22 (0.93–1.87)	NA	NA	NA	1–0.5
2a Menthone	NA	4.31 (3.05–7.23)	NA	NA	NA	NA
2b Menthone glyceryl acetal	0.19 (0.11–0.26)	0.95 (0.78–1.09)	0.79 (0.51–3.23)	0.46 (0.36–0.65)	1.22 (0.97–2.22)	WA
3a Thymol	0.38 (0.18–0.94)	0.51 (0.43–0.64)	0.60 (0.50–0.77)	0.71 (0.59–0.88)	0.49 (0.39–0.65)	0.66 (0.55–0.90)
4a α-Terpineol	0.59 (0.44–2.40)	0.45 (0.35–1.05)	0.59 (0.48–0.76)	0.56 (0.47–0.71)	0.75 (0.58–1.13)	0.62 (0.54–0.98)
5a Mugetanol	0.31 (0.16–0.44)	0.55 (0.34–0.75)	0.17 (0.03–0.29)	0.17 (0.02–0.32)	0.36 (0.12–0.57)	0.80 (0.51–1.25)
6a Pulegone	0.84 (0.57–1.22)	1.33 (0.88–2.31)	1.62 (1.19–2.45)	3.31 (2.74–5.0)	3.27 (2.59–5.43)	5.25 (4.06–10.55)
β-Caryophyllene	1.03 (0.55–2.12)	0.80 (0.42–1.20)	2.22 (1.82–2.80)	5.29 (3.55–12.59)	NA	NA

[a] Control = ethanol.
[b] Fiducial limits.
[c] NA = not active at the highest concentration tested (20 g L^{-1}).
[d] WA = weakly active (less than 50% knockdown or kill at the highest concentration tested).
[e] Compounds were purchased from Haarmann & Reimer.

than L-menthol. It was also observed that the carbon–carbon double bond increased the mosquitocidal activity in pulegone, which showed more activity than L-menthone but less activity than L-menthol.

4 CONCLUSION

This is the first report of mosquitocidal activity of menthol and its derivatives against *An. tessellatus, Cx. quinquefasciatus* and *Ae. aegypti*. Derivative synthesis followed by SAR studies identified several mosquitocidal compounds, i.e. menthyl chloroacetate, menthyl dichloroacetate, menthyl cinnamate, menthone glyceryl acetal, thymol, α-terpineol and mugetanol, which showed good activity against local disease vector mosquitoes, *An. tessellatus, Cx. quinquefasciatus* and *Ae. aegypti*. These compounds have potential to be developed as mosquitocidal compounds after toxicological evaluation for vertebrates, including humans, and ecotoxicological evaluations and commercial feasibility studies. SAR studies of L-menthol derivatives provided clear indications that minor structural and functional group variations, i.e. shape, size and degree of unsaturation, type of functional group and position of functional group and type of derivatization, can contribute towards enhancing mosquitocidal activity.

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