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Lavandula gibsoni and *Plectranthus mollis* essential oils: chemical analysis and insect control activities against *Aedes aegypti*, *Anopheles stephensi* and *Culex quinquefasciatus*

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Abstract Essential oils and acetone extracts from *Lavandula gibsoni* and *Plectranthus mollis*, family Lamiaceae, were investigated for their mosquito larvicidal activity against 4th instar larvae of *Aedes aegypti*, *Anopheles stephensi* and *Culex quinquefasciatus*. LC_{50} values against these three species were 48.3, 62.8 and 54.7 mg/L for *L. gibsoni* essential oil and 118.5, 137.2 and 128.1 mg/L, respectively, for its acetone extract, while LC_{50} values for *P. mollis* essential oil were 25.4, 33.5 and 29.5 mg/L and 195.0, 213.8 and 209.0 mg/L, respectively, for its acetone extract. Repellence of the essential oils was assessed against *A. aegypti* adults. *L. gibsoni* essential oil provided 100 % protection for more than 7 h at a concentration of 2.0 mg/cm^2. Under the same conditions, the standard repellent *N*,*N*-diethyl-*meta*-toluamide, at 0.25 mg/cm^2, provided 100 % protection for more than 8 h, while *P. mollis* essential oil was only weakly repellent. The major components from both essential oils were identified based on GC–MS analysis and linear retention indices. Our results demonstrated promising larvicidal activities of both essential oils against these mosquito species. *L. gibsoni* essential oil also showed promising repellent activity.

Keywords *Lavandula gibsoni* · *Plectranthus mollis* · Essential oil · Mosquito repellent · Mosquito larvicidal activity

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Introduction

Mosquitoes are major vectors for the transmission of malaria, filariasis, dengue fever, yellow fever and several viral diseases (Cheng et al. 2003; Pridgeon et al. 2008) with more than two billion people at risk, mostly in tropical countries (Snow et al. 2005). Personal protective measures, including repellents, are widely used to prevent the transmission of mosquito-borne diseases by minimizing the contact between humans and the mosquito vectors (Pitasawat et al. 2003). There is a growing concern about the toxic effects of synthetic pesticides to humans and other non-target organisms. Hence, environmentally friendly and biodegradable natural insecticides of plant origin have been receiving attention as an alternative for the control of arthropods of public health importance (Nathan et al. 2005).

Various plant extracts and phytochemicals including essential oils have been considered as potential sources of commercial mosquito control agents or as lead compounds for these products (Sukumar et al. 1991; Hostettmann and Potterat 1997; Adorjan and Buchbauer 2010; Fallatah and Khater 2010; Ghosh et al. 2012). They are biodegradable, and due to their multi-component nature, particularly in essential oils, extracts and purified extracts, are less prone to the development of resistance.

The genus *Lavandula* (Lamiaceae) is represented by two species in Maharashtra, India, of which *L. gibsoni* Grah. Ex Dalz. and Gibs (=*L. lawii* Wight), a medium-sized shrub with clusters of tiny violet flowers, is endemic to the Western Ghats and found on the hills of Maharashtra (Singh et al. 2001a). Previous phytochemical investigations of acetone extracts of the whole plant resulted in the isolation of aromatic monoterpenes (Patwardhan and Gupta 1983a) and oxygenated branched fatty acids (Patwardhan and Gupta 1983b). The acetone extract has been reported to exhibit ovicidal, antifeedant, antigonadal, oviposition deterrent and repellent properties against *Aedes aegypti, Phthorimaea operculella, Musca domestica, Dysdercus koenigii* and *Tribolium castaneum* (Sharma et al. 1981).

The genus *Plectranthus* (Lamiaceae) is represented by 14 species in Maharashtra, India (Singh et al. 2001b). *P. mollis*, an herb, locally called *Lal aghada*, is found throughout India and is used as a respiratory stimulant, vasoconstrictor, fever reducer, cardiac depressant and is also used for rheumatism, hemorrhage, mental retardation, snakebites as well as a general tonic (Lukhoba et al. 2006). It is also used as an insect repellent (Lukhoba et al. 2006). Pharmacologically, *P. mollis* is reported to exhibit relaxant activity on smooth and skeletal muscles as well as cytotoxic and anti-tumor promoting activities (Lukhoba et al. 2006). Antimicrobial and bronchodilatory activities of the essential oil are reported (Sharma and Ali 1966; Varma and Sharma 1963). Previous phytochemical investigations of *P. mollis* have resulted in the isolation of fatty acids from seeds (Mahmood et al. 1989) and β-sitosterol from hexane extracts of the whole plant (Desai et al. 1977). There is no work reported on the chemistry and biological activity of the essential oil of *L. gibsoni* while few reports (Shah et al. 1992; Padalia and Verma 2011) are available on the chemical composition of the essential oil of *P. mollis*.

In our programme for the development of safer, biodegradable and environmentally friendly insect control agents, we evaluated the larvicidal activity of essential oils and acetone extracts of *L. gibsoni* and *P. mollis* against three species of mosquitoes, viz. *A. aegypti*, *Anopheles stephensi* and *Culex quinquefasciatus*. Essential oils of both the species were also evaluated for mosquito repellent activity against *A. aegypti*. We report here chemical composition of essential oils of both the species as well as the results of the larvicidal and mosquito repellent bioassays.

Materials and methods

Plant material

Whole plants of *L. gibsoni* were collected in October 2008 in the Purandar Fort region, and whole plants of *P. mollis* were collected in October 2010 in the Satara–Kas region, Maharashtra. Plants were identified by taxonomist Dr. P. Tetali. Voucher specimens (No. SPJ-3 and SPJ-10 respectively) have been deposited at the Botanical Survey of India, Western Circle, Pune.

Isolation of essential oils

Roots were separated and aerial parts were cleaned in water to remove foreign material from fresh, mature whole plants. Essential oils were obtained from aerial parts by hydro-distillation using a Clevenger-type apparatus with yields of 0.14 and 0.034 % w/w for *L. gibsoni* (LGEO) and *P. mollis* (PMEO), respectively (on a fresh weight basis).

Preparation of acetone extracts

Aerial parts of *L. gibsoni* and *P. mollis* were air-dried, pulverized and extracted with distilled acetone, 6.0 L × 14 h, three times, at room temperature. The solvent was evaporated under reduced pressure to yield acetone extracts LGEA and PMEA, respectively, with yields of 2.8 % for both plants (on a dried weight basis).

Gas chromatography

The GC–FID analyses of the essential oils were carried out with Varian CP 3800 equipment using a GsBP5 capillary column (30 m length, 0.25 mm i.d., film thickness 0.25 mm). The oven temperature was programmed to rise from 50° to 260° at 3°/min, then held at 260 °C for 5 min; injector temperature was 250 °C; detector temperature was 300 °C; carrier gas was N_2 (with a flow rate of 1.0 mL/min); injection volume used was 1 μL; split ratio was 6:4. The linear retention indices (LRIs) of the constituents (Table 1) were determined relative to the retention times of a series of *n*-alkanes (C9–C38), and the relative percentages of the individual components of the oils were obtained from the GC–FID peak-area percentages.

GC–MS analyses

The GC–MS analyses of the essential oils were performed with a Perkin Elmer Clarus 500 gas chromatograph coupled to a Perkin Elmer Clarus 500 quadrupole mass spectrometer and equipped with a GsBP5 capillary column (30 m length, 0.25 mm i.d., film thickness 0.25 mm). The oven temperature was programmed to have initial hold at 50 °C for 1 min and then to rise from 50 to 280 °C at 5 °C/min, then held at 280 °C for 10 min; injector temperature was 280 °C; detector temperature was 300 °C; carrier gas was He (with a flow rate of 1.0 mL/min); injection volume used was 1 μL; mass spectra was recorded in positive electron impact mode at 70 eV.

Compound identification

The identification of the individual constituents of the essential oils was based on the comparison of their LRIs and mass spectra with those of authentic compounds by means of the NBS and NIST databases and published data on http://www.webbook.nist.gov/. The GC–FID temperature

Table 1 Composition (expressed as area %) of essential oils of aerial parts of *L. gibsoni* and *P. mollis*

Compound	LRI	*L. gibsoni*	*P. mollis*
α-Pinene	936	1.47	0.36
1-Octen-3-ol	980	2.20	0.16
β-Myrcene	993	2.78	1.06
3-Octanol	997	tr[a]	–
α-Phellandrene	1,007	0.52	0.31
δ^3-Carene	1,013	1.52	0.74
α-Terpinene	1,019	0.76	0.34
p-Cymeme	1,027	0.82	–
D- Limonene	1,031	2.30	2.83
Cis-ocimene	1,038	tr	–
Trans-ocimene	1,049	0.30	–
α- Terpinolen	1,094	22.22	–
Fenchone	1,096	–	19.19
Linalool	1,102	2.65	–
1-Octen-3-ol, acetate	1,113	1.49	–
Fenchol alpha/exo	1,118	–	1.35
3-Octanol, acetate	1,125	1.23	–
2,4-Cycloheptadien-1-one,2,6,6-trimethyl	1,145	1.35	–
Camphor	1,149	–	0.49
Borneol	1,170	–	0.54
p-Cymenol	1,189	–	1.22
Benzenemethanol, 4-(1-methylethyl)	1,189	4.52	–
α-Terpineol	1,194	0.77	–
1-Octen-3-yl-n-propionate	1,207	0.37	–
3-Isopropyleden-5-methylhex-4-ene-2-one	1,241	0.56	–
Piperitone oxide	1,265	–	23.76
Bornyl acetate	1,290	–	0.62
Thymol	1,305	10.42	–
Piperitenone oxide	1,374[b]	–	13.00
Copaene	1,383	–	2.90
β-Caryophyllene	1,411	–	10.39
Phenethyl-2-methylpropionate	1,444	0.23	–
Germacrene D	1,488	–	2.58
α-Caryophyllene	1,512	tr	–
δ-Cadinene	1,528	–	2.42
Caryophyllene oxide	1,590	1.21	–
γ-Eudesmol	1,649	–	0.87
Total		59.69	85.16

[a] Trace amounts

[b] http://www.fao.org/ag/agn/jecfa-flav/img/img/1574.gif, accessed on 16 March 2012

programme was set to a continuous gradient without any initial and during run temperature holds in order to allow accurate measurement of LRIs (Table 1).

Larvicidal assays

The standard World Health Organization (WHO) method of testing the susceptibility of mosquito larvae to insecticides (WHO 1996) was followed in all the experiments with slight modification. Larvicidal assays were carried out on early 4th instar larvae of *A. aegypti*, *C. quinquefasciatus* and *A. stephensi*. The test samples were dissolved in AnalaR grade acetone to prepare stock solutions. Ten early 4th instar larvae were introduced into a 100 mL glass beaker containing 50 mL water. A known volume of stock solution was added to the beaker to get various concentrations of test samples. A solvent control was run simultaneously. For each concentration and control, five replicates were used and each test was repeated three times. Beakers were kept at 26 ± 2 °C. Mortality was corrected using Abbott's formula when required. Mortality was analyzed by the log probit method, and lethal concentrations (LC_{50} and LC_{95}) were calculated (Table 2) using EPA probit analysis programme version 1.5. Bioneem®, a neem oil-based EC (0.03 % azadirachtin), which is commercially available, was used as a positive control for larvicidal activity.

Repellence assays

Mosquito repellent activity was assessed on the basis of the protection period offered by repellent test samples (Hebbalkar et al. 1992). For the study, 4–6-day old, blood starved, sucrose fed (0.5 M solution) females of *A. aegypti* were used.

A human hand covered with a snugly fitting polyethylene glove was introduced in a cage containing 100 hungry females. Mosquitoes were allowed to bite on the back of the hand through a muslin cloth screen placed over a small window (2 × 2 cm^2) cut out of the glove. Essential oils were applied on the muslin cloth screen at concentrations of 0.5, 1.0 and 2.0 mg/cm^2. Since the essential oil of *L. gibsoni* exhibited marked repellence, its major constituents, α-terpinolene and thymol were also tested individually at concentrations found in the essential oil, i.e. at 0.1, 0.2 and 0.4 mg/cm^2 and 0.05, 0.1 and 0.2 mg/cm^2, respectively, as well as at their ratio in the essential oil as a α-terpinolene: thymol (2:1) mixture at concentrations of 0.15, 0.3 and 0.6 mg/cm^2. Control muslin cloth screen was treated with solvent alone. After introduction of the hand covered with the glove into the mosquito cage, the number of bites received in the subsequent 5 min was counted. Where no bites occurred in the initial 5 min exposure, the test hand was exposed repeatedly every 30 min for 5 min until the time that a confirmed bite was received. The protection period was determined as the time elapsed between repellent application and the time at which a confirmed bite was observed. A control hand was placed in the cage randomly

Table 2 Lethal concentration [LC_{50} (mg/L) and LC_{95} (mg/L)] values of *L. gibsoni*, *P. mollis* essential oil, acetone extract and Bioneem® pesticide

Samples	*A. aegypti*		*A. stephensi*		*C. quinquefasciatus*	
	LC_{50} ± S.E.	LC_{95} ± S.E.	LC_{50}	LC_{95}	LC_{50}	LC_{95}
LGEO	48.32 ± 0.27 (44.07–52.39)[a]	127.28 ± 0.27 (113.91–146.15)	62.79 ± 0.66 (51.34–73.20)	129.91 ± 0.66 (106.55–185.30)	54.72 ± 0.28 (50.31–58.99)	139.77 ± 0.28 (125.32–160.17)
Regression equation	Y = −1.585 + 3.91X ± 0.50		Y = −4.367 + 5.21X ± 1.24		Y = −2.021 + 4.04X ± 0.52	
LGAE	118.45 ± 0.74 (93.28–142.69)	264.77 ± 0.74 (243.56–284.40)	137.19 ± 0.85 (107.72–170.08)	294.49 ± 0.85 (271.72–310.03)	128.14 ± 0.75 (110.23–148.64)	278.14 ± 0.75 (254.64–298.81)
Regression equation	Y = −4.76 + 4.70X ± 1.58		Y = −5.59 + 4.95X ± 1.84		Y = −5.29 + 4.88X ± 1.61	
PMEO	25.39 ± 0.39 (23.62–27.19)	57.32 ± 0.39 (50.60–67.77)	33.51 ± 0.47 (22.26–47.07)	134.55 ± 0.47 (115.81–158.28)	29.48 ± 0.84 (20.25–39.48)	88.83 ± 0.84 (59.10–104.03)
Regression equation	Y = −1.53 + 4.65 X ± 0.57		Y = 0.84 + 2.72 X ± 0.76		Y = −0.007 + 3.41 X ± 0.92	
PMAE	194.95 ± 0.61 (184.83–204.39)	341.04 ± 0.61 (313.84–382.25)	213.79 ± 0.61 (204.24–223.29)	360.95 ± 0.61 (332.84–402.75)	209.04 ± 0.63 (200.30–219.10)	350.79 ± 0.63 (324.09–382.53)
Regression equation	Y = −10.50 + 6.77X ± 1.44		Y = −11.84 + 7.23X ± 1.4		Y = −11.97 + 7.31X ± 1.48	
Bioneem®	150.8 ± 1.02 (142.09–159.145)	285.39 ± 1.02 (262.62–317.65)	79.84 ± 0.38 (74.95–85.09)	181.76 ± 0.38 (159.57–216.62)	60.26 ± 0.32 (52.67–67.10)	263.57 ± 0.32 (202.43–402.18)
Regression equation	Y = −7.938 + 5.939X ± 1.02		Y = −3.758 + 4.604X ± 0.73		Y = 0.431 + 2.566X ± 0.60	

LGEO *L. gibsoni* essential oil, *PMEO* *P. mollis* essential oil, *LGAE* *L. gibsoni* acetone extract, *PMAE* *P. mollis* acetone extract

[a] 95 % confidence intervals

Table 3 Mosquito repellent activity of essential oils of *L. gibsoni* and *P. mollis*, α-terpinolene, thymol and α-terpinolene and thymol mixture (2:1) against *A. aegypti*

Samples	Protection period offered (min)		
Concentration (mg/cm^2)	0.5	1	2
LGEO	140 ± 7.08	280 ± 12.89	435 ± 10.27
PMEO	12.0 ± 1.23	32 ± 1.00	154 ± 1.87
Concentration (mg/cm^2)	0.1	0.2	0.4
α-Terpinolene	130 ± 3.51	255 ± 5.25	310 ± 7.08
Concentration (mg/cm^2)	0.05	0.1	0.2
Thymol	70 ± 3.54	180 ± 3.27	250 ± 5.25
Concentration (mg/cm^2)	0.15	0.3	0.6
α-Terpinolen and thymol (2:1)	140 ± 3.54	265 ± 10.39	370 ± 10.02

LGEO *L. gibsoni* essential oil, *PMEO* *P. mollis* essential oil

before or after the treated hand to assess the ability of mosquitoes to bite (Table 3). *N*,*N*-diethyl-*meta*-toluamide was used as positive control.

Results

Analysis of essential oil

The identified essential oil constituents and their relative abundance are given in Table 1. The essential oil of *L. gibsoni* contained α-terpinolene (22.22 %), thymol (10.42 %) and 4-(1-methylethyl)benzenemethanol (4.52 %) as major components, and to a lesser extent, β-myrcene, linalool, limonene and 1-octen-3-ol.

Identified constituents of *P. mollis* essential oil and their relative abundance are given in Table 1. It was found to contain piperitone oxide (23.76 %), fenchone (19.19 %), piperitenone oxide (13.00 %) and β-caryophyllene (10.39 %) as major components and to a lesser extent limonene, copaene, germacrene D and δ-cadinene. Of the 85.16 % of the total components identified, monoterpenes constituted 78.28 % and sesquiterpenes 21.72 %. This oil was marked by its low percentage of aromatic compounds.

Mosquito larvicidal activity

The essential oils were evaluated for larvicidal activity against *A. aegypti*, *A. stephensi* and *C. quinquefasciatus* at concentrations ranging from 15 to 150 mg/L. Essential oil of *L. gibsoni* produced 100 % mortality at 150 mg/L against these species with LC_{50} values in the range of 48.32–62.79 mg/L (Table 2). Essential oil of *P. mollis* was more potent with 95.33 % mortality at 125 ppm against *A. stephensi*, 100 % mortality at 100 mg/L against *A. aegypti* and at 125 mg/L against *C. quinquefasciatus* with LC_{50} values in the range 25.39–33.51 mg/L. Under the same conditions, Bioneem® produced LC_{50} values in the range 60.26–150.80 mg/L. Acetone extracts were evaluated at concentrations ranging from 75 to 500 mg/L. Acetone extract of *L. gibsoni* produced 100 % mortality at 250 mg/L while that of *P. mollis* produced 100 % mortality at 500 mg/L.

Mosquito repellent activity

Since repellence assumes volatility of the active compounds, only the essential oils were evaluated for their repellent activity. The oils were evaluated against *A. aegypti* at 0.5, 1.0 and 2.0 mg/cm^2 concentrations (Table 3). At 2.0 mg/cm^2, essential oil of *L. gibsoni* offered 100 % protection for a period of 7 h 15 min (435 min) while DEET offered 100 % protection for more than 8 h (480 min) at 0.25 mg/cm^2. Essential oil of *P. mollis* offered 100 % protection from mosquito bites for 12, 32 and 154 min at 0.5, 1.0 and 2.0 mg/cm^2, respectively (Table 3).

Discussion

Essential oils of different species of genus *Lavandula* have been reported to contain linalool, linalyl acetate, 1,8-cineol, camphor, fenchol, fenchone, borneol, terpinen-4-ol, β-pinene, phenylacetaldehyde, α-phellandrene and β-phellandrene as major constituents (Iriti et al. 2006; Fiocco et al. 2011; Bousmaha et al. 2006; Ristorcelli et al. 1998; Figueiredo et al. 1995). The composition of *L. gibsoni* essential oil is thus found to be significantly different from that of other *Lavandula* species. Previous reports on mosquito larvicidal activity of essential oils from the genus *Lavandula* show weak to moderate activities. For example, oil of *L. officinalis* was reported to exhibit mortality to *A. stephensi* (LC_{50} 83.6 mg/L) (Kumar and Dutta 1987) and *L. angustifolia* to *Aedes albopictus* ($LC_{50} > 250$ mg/L). Essential oil composition of the latter was found to contain fenchone, camphor and camphene as the major constituents (Conti et al. 2010). Similarly, essential oil of *L. stoechas* exhibited toxicity to *Culex pipiens molestus* (LC_{50} 89.0 mg/L) (Traboulsi et al. 2002). Fenchone and camphor are reported as major constituents of the oil (Ristorcelli et al. 1998). α-Terpinolene and thymol, major constituents of the oil of *L. gibsoni*, are reported to show mortality to 4th instar larvae of *A. aegypti* (LC_{50} 28.4 μg/mL) and *Culex pipiens* (LC_{50} 37.95 μg/mL) (Kishore et al. 2011). Recently, an essential oil of *Plectranthus amboinicus* with thymol and carvacrol as major constituents was found to exhibit a LC_{50} value of 28.37 mg/L against larvae of *A. stephensi* (Senthilkumar and Venugopalan 2010). The potent activity of the essential oil of *L. gibsoni* thus could be due to the higher amounts of α-terpinolene and thymol. Since essential oil of *L. gibsoni* showed strong repellency and contained α-terpinolene and thymol in a 2:1 ratio, a separate study was carried out to assess the repellent activity of these compounds individually and as combination in a 2:1 ratio at concentrations found in the essential oil (Table 3). This mixture accounted for 75 % of the overall activity of the oil.

Previous analyses of the essential oil of *P. mollis* collected from Himalayan regions have reported piperitone oxide, piperitenone oxide and terpinolene as major constituents (Shah et al. 1992; Padalia and Verma 2011). The oil investigated here was obtained from *P. mollis* collected from the Western Ghats and is very similar in composition to that obtained from the above region. Piperitenone oxide, one of the major constituents of the oil of *P. mollis*, is reported to be highly active against *C. pipens* larvae with an LC_{50} value of 9.95 mg/L (Koliopoulos et al. 2010). The potent activity of the essential oil of *P. mollis* thus could be due to higher amounts of piperitenone oxide.

Acetone extracts of both the species were less active than their respective essential oils. Acetone extract of *L. gibsoni* contains ethoxylated monoterpenes, triterpenes, coumarin and flavonoids (Kulkarni and Joshi 2012). Some of these, isolated in large quantities, were evaluated for larvicidal activities, but proved to be inactive (unpublished results). Acetone extract of *P. mollis* has been found to contain sterol, triterpenes, flavonoids and phenylpropanoids (Kulkarni et al. 2012), but these constituents also lack any significant larvicidal activity.

Conclusion

Our results demonstrate promising larvicidal activity of the essential oils of both plant species against three species of mosquitoes. Acetone extracts of both species as well their constituents were weakly active. This suggests that volatile components of both plant species are the active larvicidal agents, particularly α-terpinolene and thymol in *L. gibsoni* and piperitenone oxide in *P. mollis*. Essential oil of *L. gibsoni* also exhibited potent repellent activity against *A. aegypti*; the activity was again attributable to α-terpinolene and thymol. Our study suggests a potential use of essential oil of *L. gibsoni* as a potent mosquito control agent at both larval and adult stages with oil of *P. mollis* having potential use against the larval stage. These two oils can be combined and used as a multi-component alternative to synthetic pesticides and repellents. Our study also provides the first characterization of the essential oils of *L. gibsoni* with chemical composition significantly different from essential oils of other *Lavandula* species.

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