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Evaluation of the insecticidal activity of essential oils and their mixtures against Aedes aegypti (Diptera: Culicidae)

Natalia Ríos a, Elena E. Stashenko b, Jonny E. Duque c,*

a Universidad Industrial de Santander, Escuela de Química, Centro de Investigaciones en Enfermedades Tropicales (CINTROP), Bucaramanga, Colombia
b Universidad Industrial de Santander, Escuela de Química, Centro de Investigación en Biomoléculas – CIBIMOL y Centro Nacional de Investigación para la Agroindustrialización de Plantas Aromáticas y Medicinales Tropicales – CENIVAM, Bucaramanga, Colombia
c Universidad Industrial de Santander, Escuela de Medicina, Departamento de Ciencias Básicas, Centro de Investigaciones en Enfermedades Tropicales (CINTROP), Bucaramanga, Colombia

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ABSTRACT

The search for new insecticides to control dengue fever, chikungunya, and Zika vectors has gained relevance in the past decades. The aim of the present study was to evaluate the larvicidal action of essential oils (EOs) from Thymus vulgaris, Salvia officinalis, Lippia origanoides, Eucalyptus globulus, Cymbopogon nardus, Cymbopogon martinii, Lippia alba, Pelargonium graveolens, Turnera diffusa, and Swinglea glutinosa on Aedes (Stegomyia) aegypti. The EOs were extracted by microwave-assisted hydrodistillation and characterized by gas chromatography/mass spectrometry (GC/MS). The chemical components of the EOs were identified by linear retention indices and mass spectra. Lethal concentrations (LC50 and LC90) were determined by probit analysis using larvae of Ae. aegypti between the third and the fourth instars. All EOs achieved larvicidal activity at LC50 values lower than 115 mg/L. The lowest LC50 value (45.73 mg/L) corresponded to T. vulgaris EO, whereas C. martini EO showed the highest LC50 (LC50 = 114.65 mg/L). Some EO mixtures showed lower LC50 than oils used individually, such as the mixtures of L. origanoides + S. glutinosa (LC50 = 38.40 mg/L), T. diffusa + S. glutinosa (LC50 = 63.71 mg/L), and L. alba + S. glutinosa (LC50 = 48.87 mg/L). The main compounds of the EOs with highest larvicidal activity were thymol (42%) and p-cymene (26%).

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Introduction

Several diseases such as yellow fever, dengue fever, chikungunya, and Zika fever, among several others, can be transmitted by Aedes aegypti (L., 1762) to human beings. Diseases are symptomatic manifestations of infections. Based on its morbidity and rates of mortality, dengue fever is considered the most serious disease from an epidemiological point of view. Approximately 60 million people around the world are estimated to acquire the virus each year resulting in about 10,000 deaths (Bhatt et al., 2013; Stanaway et al., 2016). In the case of Zika fever, global alarms have been activated due to the association of the virus with cases of microcephaly in newborns and Guillain-Barré syndrome reported by health institutions in Brazil and French Polynesia (Abushouk et al., 2016; Plourde and Bloch, 2016).

Due to the lack vaccines against these diseases, prevention strategies are focused on the control of larvae and adult Ae. aegypti populations. The application of synthetic insecticides (organophosphates-OP and pyrethroids-PI) is the most common approach used worldwide (Brandler et al., 2013). On the other hand, Bacillus thuringiensis var israelensis (Bti) is a bacteria widely evaluated in programs for Culicidae control. This mosquito control method is environmentally safe, commercially available and cheaper than synthetic insecticides (OP and PI). However, the principal disadvantage of using Bti in control programs is the low persistence in field conditions (Ritchie et al., 2010; Boyce et al., 2013; Moshi and Matoju, 2017).

Several studies have been conducted to identify new insecticides obtained from secondary metabolites of aromatic and medicinal plants, seeking effective alternatives to combat vector mosquitoes. The aim of such studies is to discover options to replace traditional chemical insecticides and determine natural ingredients to make formulations that can be used in the design of new insecticides (Carreño et al., 2014).

Compared to synthetic products, natural pesticides are less harmful to human health and ecosystems, and so they are widely accepted by the general population. Despite these benefits, commercial insecticides still have more effective lethal concentrations...
(LC), lethal doses (LD) and lethal times (LT) than natural products (Shaalan et al., 2005; Koul et al., 2008). Therefore, it is important to characterize the insect-killing effectiveness of essential oils (EO) or plant extracts (PE) in their first screening phase in order to determine their promise as insecticides. One of the criteria to guide new larvicide research is that the candidate substances have an LC₅₀ < 100 mg/L (Cheng et al., 2003; Dias and Moraes, 2014). However, this criterion does not include important aspects of the control and protection against mosquito bites, such as repellency, deterrence, and attraction (Castillo et al., 2017).

More studies are needed to compare the insecticidal action of EOs and PEs obtained at different geographical locations (Amer and Mehlhorn, 2006a; Pavela, 2008; Caballero-Gallardo et al., 2012; Manimaran et al., 2012). It is also important to understand that the chemical composition of an EO or PE can determine its insecticidal effect, and that this may vary intra- and interspecifically, according to soil, plant anatomy, edaphic factors, and environmental conditions (Bakkali et al., 2008; Dias and Moraes, 2014). Based on these premises, the aim of the present study was to evaluate the insecticidal activity of essential oils isolated from different aromatic plants, as follows: Salvia officinalis (Lamiaceae), Thymus vulgaris (Labiatae), Eucalyptus globulus (Myrtaceae), Lippia alba (Verbenaceae), Turnera diffusa (Turneraceae), Pelargonium graveolens (Geraniaceae), Cymbopogon nardus, and Cymbopogon martini (Poaceae), Swinglea glutinosa (Rutaceae), as well as two different chemotypes of Lippia organoides (phellandrene and thymol).

Material and methods

The experiments were developed using Ae. aegypti insects from the Rockefeller colony. Mosquitoes were kept in 40 × 40 × 40 cm breeding cages under special conditions of humidity (70 ± 5%), photoperiod (12:12), and temperature (25 ± 5 °C). Female mosquitoes were fed with Wistar rat blood (the US ethics committee was previously informed, as stated in CEINCI-UIS Minute No. 3, 2013; male mosquitoes were fed with 10% sucrose solution.

Essential oil isolation

The plants were collected from fields located in Santander, Colombia (Table 1). The EOs were extracted by microwave-assisted hydrodistillation (MWHD) as described by Stashenko et al. (2004). In the case of MWHD, plant material and the water were heated using a domestic microwave oven (2.45 GHz, 800 W), modified with a lateral orifice to connect the flask and the condenser. The microwave oven worked at full power (800 W) for 30 min (10 min × 3). The EO was collected in a Dean-Stark, and finally, the condensate was decanted and dried with anhydrous sodium sulfate.

The EOs were characterized by gas chromatography/mass spectrometry (GC/MS), using an Agilent Technologies 6890 (AT, Palo Alto, CA, USA) gas chromatograph with a DB-5MS capillary column (60 m × 0.25 mm id × 0.25 mm df) using helium (99.995% purity) as carrier gas at a flow rate of 1 mL/min and an Agilent Technologies 5973 mass selective detector. Ionization was used electron energy achieved at 70 eV. The temperatures of the injector and the transfer line were set at 285 and 250 °C, respectively. The initial column temperature was 50 °C, which was increased by 3 °C/min up to 150 °C, and the 250 °C temperature was finally reached at 10 °C/min. The major components of the EOs were identified using the linear retention indices and mass spectra, which were compared with those from the NIST, Wiley, and ADAMS databases (Stashenko et al., 2004).

Insecticidal activity

Experiments were initially conducted at exploratory concentrations (EC) of EO with larvae of Ae. aegypti between the third and the fourth instars. Larvae were placed in 100 mL plastic cups containing a solution of EO and mineral water. Mortality rates between 2 and 98% have been previously found after exposing larvae to EC of essential oils (Aciole et al., 2011; Vera et al., 2014). The concentrations being tested were initially 30, 300, and 1000 mg/L. Each treatment was repeated four times (N = 120 larvae), and experiments were replicated three times on different days. The control test used dimethyl sulfoxide (DMSO, 0.5%) and mineral water. Larval counts were performed at 24 and 48 h after initial exposure to each EO concentration. The criteria to consider larvae as dead were that the individuals lacked all movement and failed to reach the water surface (WHO, 1996). Values of LC₅₀, LC₉₀, and mortality rates were determined for five selected EOs. The results of mortality and survival bioassays were subjected to Probit analysis (Finney, 1971).

Results

The EOs obtained by MWHD presented different extraction yields. E. globulus was the plant from which the highest amount of EO was obtained (2.0%, w/w). The major components in the oil were thymol (T. vulgaris), 1,8-cineole (S. officinalis), limonene (L. organoides chemotype-phellandrene), thymol (L. organoides, thymol chemotype), 1,8-cineol (E. globulus), citronellol (C. nardus), geraniol (C. martini), carvone (L. alba), drima-7,9(11)-diene (T. diffusa), and citronellol in the EO of P. graveolens (Table 2).

All EOs displayed insecticidal action against Ae. aegypti larvae at 24 and 48 h (Table 3). The relationship between concentration and mortality was most effective with the oil mixture composed of L. organoides and S. glutinosa (38.40 mg/L). T. vulgaris EO showed the

<table>
<thead>
<tr>
<th>Scientific name</th>
<th>Family</th>
<th>Common name</th>
<th>Voucher No.</th>
<th>Site of collection</th>
<th>EO yield, % (p/p)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thymus vulgaris</td>
<td>Labiatae</td>
<td>Thyme</td>
<td>555843</td>
<td>Sucre, Santander</td>
<td>0.3</td>
</tr>
<tr>
<td>Salvia officinalis</td>
<td>Lamiaceae</td>
<td>Garden sage</td>
<td>555844</td>
<td>Sucre, Santander</td>
<td>0.4</td>
</tr>
<tr>
<td>Lippia organoides (Phellandrene)</td>
<td>Verbenaceae</td>
<td>Wild oregano</td>
<td>519798</td>
<td>Cenivam, Bucaramanga</td>
<td>0.4</td>
</tr>
<tr>
<td>Lippia organoides (thymol)</td>
<td>Verbenaceae</td>
<td>Wild oregano</td>
<td>519799</td>
<td>Cenivam, Bucaramanga</td>
<td>1.6</td>
</tr>
<tr>
<td>Eucalyptus globulus</td>
<td>Myrtaceae</td>
<td>Blue gum</td>
<td>C-470</td>
<td>Cenivam, Bucaramanga</td>
<td>2.0</td>
</tr>
<tr>
<td>Cymbopogon nardus</td>
<td>Poaceae</td>
<td>Citronella grass</td>
<td>578357</td>
<td>Cenivam, Bucaramanga</td>
<td>0.4</td>
</tr>
<tr>
<td>Cymbopogon martini</td>
<td>Poaceae</td>
<td>Gingergrass</td>
<td>587116</td>
<td>Cenivam, Bucaramanga</td>
<td>0.4</td>
</tr>
<tr>
<td>Lippia alba</td>
<td>Verbenaceae</td>
<td>Quick relief</td>
<td>480750</td>
<td>Cenivam, Bucaramanga</td>
<td>0.5</td>
</tr>
<tr>
<td>Turnera diffusa</td>
<td>Turneraceae</td>
<td>Damiana</td>
<td>516293</td>
<td>Los Santos, Santander</td>
<td>0.7</td>
</tr>
<tr>
<td>Pelargonium graveolens</td>
<td>Geraniaceae</td>
<td>Wildagall</td>
<td>51718</td>
<td>Cenivam, Bucaramanga</td>
<td>0.2</td>
</tr>
<tr>
<td>Swinglea glutinosa</td>
<td>Rutaceae</td>
<td>African lemon</td>
<td>521530</td>
<td>Cenivam, Bucaramanga</td>
<td>0.2</td>
</tr>
</tbody>
</table>

Table 1
Essential oil yields collection sites and registration numbers (voucher) of plants studied in this work.
Table 2
Percentages of major components in the essential oils studied.

<table>
<thead>
<tr>
<th>Plant</th>
<th>Major components (%)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>T. vulgaris</td>
<td>Thymol (42.0), p-cymene (26.4), γ-terpinene (6.3), linalool (2.9), trans-β-carophyllene (2.6)</td>
<td>Unpublished data</td>
</tr>
<tr>
<td>S. officinalis</td>
<td>1,8-Cineol (26.6), α-thujone (18.1), trans-β-carophyllene (7.3), α-humulene (5.4)</td>
<td>Unpublished data</td>
</tr>
<tr>
<td>L. origanoides</td>
<td>Limonene (15.0), p-cymene (14.6), α-phellandrene (10.3), trans-β-carophyllene (5.8), α-humulene (2.9), α-pinene (2.5), γ-terpinene (2.1)</td>
<td>(Stashenko et al., 2010)</td>
</tr>
<tr>
<td>L. origanoides (phellandrene)</td>
<td>Thymol (66.1), p-cymene (7.2), γ-terpinene (4.4), trans-β-carophyllene (3.6), α-humulene (2.4), methyl thymol ether (2.3), thymol acetate (2.0), α-thujone (1.0)</td>
<td>(Stashenko et al., 2010)</td>
</tr>
<tr>
<td>E. globulus</td>
<td>1,8-Cineol (69.4), α-pinene (4.6), viridiflorol (4.1), α-terpenyl acetate (3.3), limonene (3.0)</td>
<td>Unpublished data</td>
</tr>
<tr>
<td>C. nardus</td>
<td>Citronellal (21.8), citronellol (18.1), geranial (11.3), germacrene D (4.6), linalol (3.5)</td>
<td>Unpublished data</td>
</tr>
<tr>
<td>C. martini</td>
<td>Geraniol (83.9), geranial acetate (9.2), linalool (2.3), trans-β-carophyllene (1.0)</td>
<td>(Rodríguez et al., 2012)</td>
</tr>
<tr>
<td>L. alba (carvone)</td>
<td>Carvone (35.3), limonene (35.0), bicyclosquephellandrene (9.6), piperitene (3.4), piperitone (1.0)</td>
<td>(Aguédo-Gomez et al., 2010)</td>
</tr>
<tr>
<td>T. diffusa</td>
<td>Drima-7,9(11)-diene (22.9), β-viridiflorene (6.6), α-silene (5.9), valencene (5.5), trans-β-carophyllene (5.2), trans-murola-4(14)-diene (5.2), p-cymene (2.1)</td>
<td>Unpublished data</td>
</tr>
<tr>
<td>P. graveolens</td>
<td>Citronellol (14.9), geranial (8.4), geranial (7.5), guaiene (7.4), germacrene D (3.7), iso-methone (3.7), geranyl formate (3.2)</td>
<td>Unpublished data</td>
</tr>
<tr>
<td>S. glutinosa</td>
<td>trans-Nerolidol (28.4), germacrene D (20.5), α-pinene (9.1), trans-β-carophyllene (7.5), δ-elemene (3.6), α-cadinol (2.1), γ-elemene (2.6), β-Cadinen (2.0), espatenol (1.7), α-humulene (1.5), β-elemene (1.3), geranyl acetate (1.0)</td>
<td>(Stashenko et al., 2015)</td>
</tr>
</tbody>
</table>

Table 4
Larvicidal activity (in mg/mL) of the different EOs against Ae. aegypti larvae at 24 and 48 h.

<table>
<thead>
<tr>
<th>Essential oil or mixture</th>
<th>24 h</th>
<th>48 h</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>LC50</td>
<td>LC50</td>
</tr>
<tr>
<td>(L. origanoides + S. glutinosa)</td>
<td>38.40 (35.52–42.37)</td>
<td>94.91 (77.38–128.56)</td>
</tr>
<tr>
<td>T. vulgaris</td>
<td>45.73 (41.29–53.80)</td>
<td>96.25 (75.01–149.01)</td>
</tr>
<tr>
<td>(S. glutinosa + L. alba)</td>
<td>48.87 (46.17–51.50)</td>
<td>101.76 (92.02–116.32)</td>
</tr>
<tr>
<td>L. origanoides (Phellandrene)</td>
<td>53.79 (50.90–56.69)</td>
<td>116.60 (102.56–140.31)</td>
</tr>
<tr>
<td>L. origanoides (Thymol)</td>
<td>56.18 (53.30–59.89)</td>
<td>124.55 (105.55–160.30)</td>
</tr>
<tr>
<td>(T. diffusa + S. glutinosa)</td>
<td>63.71 (60.75–67.71)</td>
<td>117.70 (103.39–141.81)</td>
</tr>
<tr>
<td>L. alba (Carvone)</td>
<td>72.34 (69.87–75.05)</td>
<td>110.84 (102.69–123.28)</td>
</tr>
<tr>
<td>S. officinalis</td>
<td>76.43 (71.84–83.79)</td>
<td>123.92 (106.98–136.75)</td>
</tr>
<tr>
<td>C. nardus</td>
<td>75.85 (69.15–86.82)</td>
<td>219.68 (160.93–345.02)</td>
</tr>
<tr>
<td>E. globulus</td>
<td>92.55 (89.37–97.00)</td>
<td>136.82 (124.67–157.14)</td>
</tr>
<tr>
<td>P. graveolens</td>
<td>108.96 (103.62–115.74)</td>
<td>176.61 (157.84–208.81)</td>
</tr>
<tr>
<td>C. martini</td>
<td>114.65 (107.26–124.94)</td>
<td>251.26 (211.65–321.05)</td>
</tr>
</tbody>
</table>

LC50 is the lethal concentration causing mortality of 50% of organisms exposed to treatment. LC50 is the lethal concentration causing mortality of 50% of organisms exposed to treatment. The confidence interval is given in parentheses. The statistical analysis was well adjusted to the probit model (Finney, 1947).

The L. origanoides EOs of phellandrene and thymol chemo-
types, presented similar insecticidal effects (LC50 = 53.79 mg/L and LC50 = 56.18 mg/L, respectively), which matches the LC50 of L. origanoides, obtained by Vera et al. (2014) with Ae. aegypti.

Despite their different major compounds, the larvicidal effect of EOs from L. alba, C. nardus, and S. officinalis showed similar LC50 values (Table 3). In the case of L. alba, the LC50 value (72.34 mg/L) was higher than that found by Vera et al. (2014) with Ae. aegypti (LC50 = 44.26 mg/L). This lower activity could be related to a lower amount of carvone in the EO (35.3%) as compared to a previous report (38.3%) by Vera et al. (2014), who found an amount of 38.3%. Besides L. alba insecticidal action, the plant has a record as a repellent with other insects, such as Tribolium castaneum (Olivero-Verbel et al., 2013).

In the present study, the EO of C. nardus presented a much more effective larvicidal activity against Ae. aegypti than that reported by Tennyson et al. (2013) in India (1374.05 mg/L). On the other hand, we obtained lower LC50 values than those obtained by Manimaran et al. (2012) for Ae. aegypti (LC50 = 47.21) and Anopheles stephensi (47.61 mg/L); the EOs in that study were obtained from plants cultivated in India.

Pavela (2008) reported a LC50 of 159 mg/L with Cx. quinquefasciatus larvae in a study on S. officinalis, a plant of Eurasian origin; when we compared those results with our results on Ae. aegypti, we observed that the LC50 was lower (76.43 mg/L), indicating that the EO of this plant had higher insecticidal activity than S. officinalis extract.

Discussion

All of the studied EOs, both individually and as mixtures, presented insecticidal activity against Ae. aegypti larvae. Only C. martini and P. graveolens presented an LC50 > 100 mg/mL, indicating that all EOs evaluated in this study can be utilized as good candidates for the design of new mosquito insecticides against mosquito control (Dias and Moraes, 2014). The mixture of L. origanoides and S. glutinosa was proven to cause the highest insect mortality (LC50 = 38.40 mg/L). As shown by Vera et al. (2014), the mixtures of EOs, in this case L. origanoides (53.37 mg/L) and S. glutinosa (65.71 mg/L), may enhance the toxic effect of individual oils on Ae. aegypti larvae.

Our results showed T. vulgaris to have the best larvicidal action (LC50 = 45.73 mg/L). This bioactivity reflects a study by Massebo et al. (2009), who studied EO extracted from leaves and seeds of plants from Ethiopia, yet the LC50 was lower (17.3 mg/L). Also, T. vulgaris extracts from plants grown in the Czech Republic (LC50 = 48 mg/L) with Culex quinquefasciatus (Pavela, 2008) confirm our present results. Thymol and p-cymene were the major compounds identified in this plant, and the toxicity against mosquitoes was consistent with other reports on these metabolites (Dias and Moraes, 2014).
The EOIs of *E. globulus* (LC50 = 92.55 mg/L), *P. graveolens* (LC50 = 108.96 mg/L), and *C. martini* (LC50 = 114.65 mg/L) showed less effectiveness. Based on the criterion of plants with LC50 < 100, only *E. globulus* EO would be promising as an insecticide (Dias and Moraes, 2014). The EO of this plant had the greatest yield (2.0%), w/w, and mortality rates of 32.92% at 24 h and 34.17% at 48 h were achieved with a concentration of 93 mg/L (Table 3). These results are consistent with those presented by Amer and Mehnhorn (2006b), who reported *Aedes* larval mortality rates from 16.7% with EO solutions (50 mg/L) at 24 h of treatment.

*L. organoides* and *S. glutinosa* mixture showed the highest larvicidal activity (LC50 = 38.40 mg/L) of the three mixtures analyzed in this study. It should be highlighted that these EOIs, separately, had higher LC50s than when evaluated as part of mixtures, as has been observed with EOIs of *L. organoides* (LC50 = 53.79 mg/L) and *S. glutinosa* (LC50 = 65.71 mg/L) (Vera et al., 2014). These data indicate that the insecticidal effect of EOIs can be potentiated by using mixtures of EO, probably due to a synergic effect (Mansour et al., 2015).

Although there is extensive information on botanical products such as essential oils and plant extracts for mosquito control (larval and adults), it is unusual to find them in formulations of commercial insecticides. Plants such as *Azadirachta indica* and *Melia azedarach* (Melaceae) are among the few that are part of commercial biopesticides. These two species of plants have been studied on at least 103 species of insects and have eco-friendly effects (Mazid, 2011; Thangavel and Sridevi, 2015; Moshi and Matoujo, 2017). However, the insecticidal effect on mosquito larvae of these plants is not so effective (LC50 > 1 x 10^{-4} mg/L) as that of essential oils (LC50 < 50 mg/L) (Howard et al., 2009; Kishore et al., 2011; Dias and Moraes, 2014; Vera et al., 2014). This is a good reason to use the plants presented here as source of ingredients for design new insecticides.

**Conclusion**

All of the EOIs evaluated in the present study showed insecticidal activity. The EO of *T. vulgaris* and the mixture of *L. organoides* and *S. glutinosa* showed highest larvicidal action on *Ae. aegypti*. The main compounds of the EOIs with higher larvicidal activity were thymol (42%) and p-cymene (26.4%).

**Conflicts of interest**

The authors declare no conflicts of interest.

**Acknowledgments**

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