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Full Length Research Paper

Evaluation of larvicidal activity of the essential oils of plants species from Brazil against *Aedes aegypti* (Diptera: Culicidae)

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Essential oils obtained from the leaves of *Myrcia ovata* Cambess.*, Psidium guajava* L., *Spondias purpurea* L. and *Plectranthus amboinicus* (Lour.) Spreng. were gotten by hydrodistillation, analyzed by GC-MS and GC, and tested against third-instar *Aedes aegypti* larvae. The essential oils exhibited significant larvicidal activity with LC_{50} values ranging from 24.7 to 192.1 µg/ml. Larvicidal activities of 1,8-cineole and carvacrol, the major compounds of the essential oil from *P. guajava and P. amboinicus* were also evaluated and their LC_{50} values were 47.9 ± 0.3 and 58.9 ± 0.4 µg/ml, respectively. The results of this study showed that these essential oils and their major compounds may be potent source of natural larvicides.

Key words: *Myrcia ovata*, *Psidium guajava*, *Spondias purpurea*, *Plectranthus amboinicus*, larvicidal activity, *Aedes aegypti*.

INTRODUCTION

Mosquito-borne diseases cause significant morbidity, mortality and economic burden to humankind (Massebo et al., 2009). The mosquito, *Aedes aegypti* is the major vector of yellow fever, dengue and dengue hemorrhagic fever (DHF). These mosquito-borne infections are found in tropical and sub-tropical regions around the world, predominantly in urban areas and semi-urban areas. The global incidence of dengue has grown dramatically around the world in recent decades and there are approximately 2.5 billion people at risk (World Health Organization, 2009). Recently, in Brazil, the incidence of dengue has increased significantly. A total of 4,243,049 dengue cases have been reported between 1981 and 2006, including 5,817 cases of dengue hemorrhagic fever/dengue shock syndrome (DHF/DSS) and a total of 338 fatal cases (Nogueira et al., 2007).

There is currently no available vaccine or drug treatment for dengue and, at present, the only method of preventing transmission is by controlling its vector

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(Chung et al., 2009). The control of this vector is based on the destruction of breeding sites by using synthetic insecticides. However, the continued use of synthetic insecticides has resulted in resistance in mosquitoes (Melo-Santos et al., 2010). In addition, synthetic insecticides are toxic and affect the environment by contaminating soil, water and air (Dharmagadda et al., 2005), then natural products may be an alternative to synthetic insecticides because they are effective, biodegradable, eco-friendly and safe to environment (Sreelatha et al., 2010).

Essential oils have been reported to possess larvicidal activity against *A. aegypti* (Santos et al., 2006, 2007; Arriaga et al., 2007; Cheng et al., 2009; Chung et al., 2009; Feitosa et al., 2009; Aguiar et al., 2010; Magalhães et al., 2010) and since there are no reports on the larvicidal potential of *Myrcia ovata* Cambess. (Myrtaceae), *Psidium guajava* L. (Myrtaceae), *Spondias purpurea* L. (Anacardiaceae) and *Plectranthus amboinicus* (Lour.) Spreng. (Lamiaceae), the larvicidal activities of the essential oils from these species were evaluated by measurement of their LC_{50} and were also studied qualitatively and quantitatively by GC and GC-MS.

MATERIALS AND METHODS

The leaves of *M. ovata* Cambess were collected in October 2004, in Guaramiranga, State of Ceará, northeast Brazil, the leaves of *P. guajava* L. and *S. purpurea* L. were collected in June 2009, in Fortaleza, State of Ceará, Brazil, while the leaves of *P. amboinicus* (Lour.) Spreng. were collected in march 2009, from the medicinal plants garden, Horto de Plantas Medicinais, Universidade Federal do Ceará, Brazil. The plants were identified by Prof. E. P. Nunes from the Herbário Prisco Bezerra (EAC), Universidade Federal do Ceará, Brazil where voucher specimens were deposited under the numbers EAC039558 (*M. ovata*), EAC24504 (*P. guajava*), EAC 48863 (*S. purpurea*) and EAC40080 (*P. amboinicus*).

Extraction of the essential oils

Fresh leaves of all specimens were separately subjected to hydrodistillation for 2 h in a Clevenger-type apparatus. The obtained essential oils were dried over anhydrous sodium sulfate and stored under refrigeration until tested.

Analysis of the essential oils

GC analysis was performed on a Shimadzu GC-17A gas chromatograph equipped with flame ionization detector using a non-polar DB-5 fused silica capillary column (30 m × 0.25 mm× 0.25 µm film thickness). Hydrogen was used as carrier gas at a flow rate of 1 ml/min and 30 psi inlet pressure; split ratio 1:30. The column temperature was programmed from 35 to 180°C at a rate of 4°C/min, then heated at a rate of 17°C/min to 280°C and held isothermal for 10 min; both injector and detector temperatures were 250°C.

The GC/MS analysis was carried out on a Hewlett-Packard Model 5971 GC/MS instrument, using a non-polar DB-5 fused silica capillary column (30 m × 0.25 mm × 0.1 µm film thickness); carrier gas helium, flow rate 1 ml/min and with split mode. The injector and detector temperatures were 250 and 200°C, respectively. The column temperature was programmed from 35 to 180°C at 4°C/min and then 180 to 250°C at 10°C/min. Mass spectra were recorded from 30 to 450 m/z.

Individual components were identified by matching their 70 eV mass spectra with those of the spectrometer database using the Wiley L-built library and two other computer libraries, MS searches using retention indices as a pre-selection routine (Alencar et al., 1984, 1990), as well as by visual comparison of the fragmentation patterns with those reported in the literature (Adams, 2007).

Larvicidal bioassay

Aliquots of the essential oils, 1,8-cineole and carvacrol (12.5 to 500 µg/ml) were placed in a beaker (50 ml) and dissolved in H_2O/DMSO 1.5% (20 ml). Fifty third-instar *A. aegypti* larvae were delivered to each beaker. After 24 h, at room temperature, the number of dead larvae was counted and the lethal percentage calculated. A control using H_2O/DMSO 1.5% was carried out in parallel. For each sample, three independent experiments were run (Oliveira et al., 2002). The LC_{50} values of all tested essential oils, 1,8-cineole and carvacrol were calculated using the probit analysis (Finney, 1971).

Isolation and purification of 1,8-cineole and carvacrol

The monoterpenes: 1,8-cineole and carvacol were obtained from the leaves' oils after separation by column chromatography, using hexane and hexane : ethyl acetate 98:02 as eluents, respectively.

RESULTS

The leaves essential oils from *M. ovata*, *P. guajava*, *S. purpurea* and *Plectranthus amboinicus* were analyzed by gas chromatography (GC) and gas chromatography coupled with mass spectrometry (GC/MS). The chemical composition of the oils, including the retention index and the percentage relative of each constituent identified is shown in Table 1.

In searching for new strategies to control *A. aegypti* propagation, four essential oils obtained were investigated for their larvicidal activities against third-instar *A. aegypti* larvae and the results are presented in Table 2. The LC_{50} values of the essential oils ranged from 24.7 to 192.1 µg/ml.

DISCUSSION

The oils composition of leaves of *M. ovata* (Cândido et al., 2010), *P. guajava* (Santos et al., 1998; Ogunwande et al., 2003; Adam et al., 2011), *S. purpurea* (Lemos et al., 1995) and *P. amboinicus* (Vera et al., 1993; Murthy et al., 2009) have been previously reported. However, the oils of *P. guajava*, *S. purpurea* and *P. amboinicus* showed different chemical compositions. In the present study, the major constituent identified in the essential oil of *P. guajava* was 1,8-cineole (48.8%), whereas the literature reports α-pinene (65.4%) (Santos et al., 1998), limonene

Table 1. Volatile components identified in the essential oils of leaves of *M. ovata, P. guajava*, *S. purpurea* and *P. amboinicus*.

Compound	Relative content (%)				
	IK	*M. ovata*	*P. guajava*	*S. purpurea*	*P. amboinicus*
Benzaldehyde	0960	-	3.1	-	-
1,8-Cineole	1031	4.8	48.8	-	-
α-Terpineol	1189	1.1	0.3	0.7	-
Neral	1238	35.8	-	-	-
Geranial	1267	50.4	-	-	-
Carvacrol	1298	-	-	-	92.3
α -Copaene	1377	-	0,1	-	-
β-Caryophyllene	1419	-	25.6	-	3.2
α -Humulene	1455	-	3.6	-	-
γ-Muurolene	1479	-	-	6.3	-
γ-Amorphene	1495	-	-	2.2	-
α-Muurolene	1500	-	-	4.7	-
γ-Cadinene	1513	-	-	2.5	-
trans-Calamenene	1522	-	-	3.9	-
δ-Cadinene	1523	-	0.8	-	-
Caryophyllene oxide	1583	-	-	23.7	1.0
Junenol	1619	-	-	10.9	-
epi-α-Muurolol	1642	-	-	11.8	-
α-Cadinol	1654	-	-	22.9	-
cis-Calamenen-10-ol	1661	-	-	2.9	-
Total identified		92.1	82.3	92.5	96.5

Table 2. Lethal concentration values of essential oils of leaves of *M. ovata, P. guajava*, *S. purpurea* and *P. amboinicus* in 24 h.

Essential oil and compound	LC_{50} (µg/ml)
M. ovata	192.1 ± 2.1
P. guajava	24.7 ± 1.9
S. purpurea	39.7 ± 1.8
P. amboinicus	51.8 ± 0.6
Carvacrol	58.9 ± 0.4
1,8-Cineole	47.9 ± 0.3

(42.1%) (Ogunwande et al., 2003) and β-caryophyllene (18.3%) (Adam et al., 2011) as the major constituents. The different components of essential oil from *S. purpurea* were caryophyllene oxide, α-cadinol, *epi*-α-muurolol, junenol, γ-muurolene and *trans*-calamenene, Three components have been characterized (Table 1) in the essential oil of *P. amboinicus*. Carvacrol was present in 92.3%, with other components being β-caryophyllene (3.2%) and caryophyllene oxide (1.0%). An earlier report on composition of essential oil of *P. amboinicus* also mentioned carvacrol to be the major (70%) component (Murthy et al., 2009). However, in one report (Vera et al., 1993), the major components are δ-3-carene (16.3%), carvacrol (13.4%), camphor (12.3%) and γ-terpinene (11.9%). The variations in the chemical composition of essential oils can be attributed to different geographic regions where the plant species were collected. The differences in the toxicity of essential oils tested against *A. aegypti* are due to both qualitative and quantitative variations of the components.

The experiments showed that the leaves essential oil of *P. guajava* was the most potent larvicidal, with LC_{50} value of 24.7 ± 1.9 µg/ml. The oxygenated monoterpene 1,8-cineole was the major constituent of the essential oil of leaves of *P. guajava*. This compound was isolated by column chromatography and characterized by comparison of its spectral data with the values reported in the literature (Adams, 2007; Rahmnn and Ahmad, 1992).

1,8-Cineole was tested against *A. aegypti* larvae under identical conditions to compare its activity with that of the investigated essential oil, and it showed LC_{50} value of 47.9 ± 0.3 µg/ml (Table 2). Therefore, this compound is probably the active principle responsible for *P. guajava* larvicidal effect. A previous study showed that the essential oil of leaves of *Hyptis martiusii*, which showed 1,8-cineole as one of the major constituents, showed good activity against *A. aegypti* (Araújo et al., 2003).

The oil of *S. purpure*a contains high proportion of oxygenated sesquiterpenes (72.2%) (Table 1) and in general, oils containing relatively high proportions of oxygenated sesquiterpenes have stronger larvicidal activity when compared to essential oils rich in sesquiterpene hydrocarbons (Arriaga et al., 2008; Aguiar et al., 2010). Thus, the potential larvicidal of *S. purpurea* oil could be attributed to its relatively high proportions of oxygenated sesquiterpenes.

The major constituent in the essential oil of *P. amboinicus*, carvacrol, was isolated by column chromatography and characterized by comparison of its spectral data with the values reported in the literature (Adams, 2007; Rahmnn and Ahmad, 1992). This compound exhibited LC_{50} value of 58.9 ± 0.4 µg/ml (Table 2). Thus, carvacrol is probably the active compound responsible for the larvicidal activity of this oil. Several studies have shown that carvacrol possesses significant larvicidal activity against *A. aegypti* (Santiago et al., 2006; Lima et al., 2008; Silva et al., 2008).

The essential oil of *M. ovata*, which contains neral (35.8%) and geranial (50.4%) as the major constituents (Table 1), showed the weakest larvicidal activity among the oils evaluated, with LC_{50} value of 192.1 ± 2.1 µg/ml (Table 2). Similarly, Albuquerque et al. (2007) found that the essential oil of *Pectis apodocephala*, rich in neral and geranial, was toxic against *A. aegypti* larvae, with LC_{50} value of 195.0 ± 1.7 µg/ml. The literature reports that neral and geranial, isolated from the bark of *Magnolia salicifolia*, showed 100% mortality on four-instar *Aedes aegypti* larvae at 100 ppm in 24 h (Kelm et al., 1997). Therefore, the larvicidal activity of the essential oil of *M. ovata* could be attributed to these compounds. Although, caryophyllene oxide, the main constituent of essential oil of *S. purpurea*, has not been tested by us, Silva et al. (2008) reported that this compound exhibited high larvicidal activity (LC_{50} 125 ± 2.05 ppm). Therefore, this compound might be one of the compounds responsible for the larvicidal activity of essential oil of *S. purpurea*.

To our knowledge, this is the first report on the potential of the essential oils of *M. ovata, P. guajava*, *S. purpurea* and *P. amboinicus* on *A. aegypti* larvicide. These results may be useful in the search for discovering newer, more selective and biodegradable larvicidal compounds.

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