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Essential oils from *Alpinia purpurata* (Zingiberaceae): Chemical composition, oviposition deterrence, larvicidal and antibacterial activity

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ABSTRACT

Essential oils and aqueous extracts were obtained by hydrodistillation of flowers of red and pink variants of *Alpinia purpurata* (Zingiberaceae), a widely cultivated tropical ornamental plant. GC/MS analyses revealed the presence of 42 essential oil components with α-pinene, β-pinene and β-caryophyllene being the major constituents. Oils from the red and pink variants presented potent larvicidal activities against 4th instar *Aedes aegypti* with LC_{50} values of 80.7 and 71.5 ppm, respectively. Aqueous extracts of the two variants were also active against the dengue mosquito larvae (LC_{50} values of 18.3 and 12.6%, respectively). Oils and aqueous extracts of both variants showed significant oviposition deterrent effects at concentrations of 100 ppm and 20%, respectively. Oil from the red cultivar inhibited significantly the growth of gram-positive and gram-negative bacteria. The study demonstrates that products with valuable biological activities can be produced from flowers of *A. purpurata* that are not of commercial quality.

1. Introduction

The family Zingiberaceae comprises more than 1200 species that are native to tropical regions, and many of these are valued as ornamentals or employed as raw materials in the production of fibre, paper, dyes, foods, spices and perfumes. *Alpinia* is the largest genus of the family with more than 200 species (Albuquerque and Neves, 2004), several of which possess important biological activities. *Alpinia zerumbet*, for example, has been shown to act as an anti-hypertensive in rats (Lahlou et al., 2003) and as an inhibitor of HIV-1 integrase and neuroaminidase *in vitro* (Upadhyay et al., 2011). Furthermore, extracts of the rhizomes of *A. galanga* exert significant larvicidal activities against *Plutella xylostella* (Dadang and Ohsawa, 2001), while the essential oil from *Alpinia speciosa* has been found to be active against mosquito larvae (Ho, 2010). The aqueous and methanolic extracts of the seeds and leaves of *A. speciosa* are also reported to possess antioxidant and antimicrobial activities and to inhibit tyrosinase (Ho, 2010).

Alpinia purpurata (Viell.) K. Schum. is an ornamental plant that is native to the Pacific Islands but is currently cultivated on a large scale in the state of Pernambuco, Brazil, by virtue of its capacity to flower continuously throughout the year and of the exuberance and durability of the blooms so-produced. However, only the highest quality blooms can be commercialised and those that fall short of this standard are discarded as waste material. Although information is available concerning the composition of the essential oil derived from flowers of *A. purpurata* (Zoghbi et al., 1999; Victório et al., 2010), the pharmacological activities of extracts of this species have received little attention. It is known, however, that essential oils in general exhibit a wide variety of biological effects including antibacterial, antiviral, antifungal and antiparasitic activities.

With the objective of establishing potential applications for the residual material from the large-scale harvesting of flowers of *A. purpurata*, we have investigated the biological activities of the essential oil derived from discarded floral material against *Aedes aegypti*. This mosquito is widely distributed in tropical and subtropical areas throughout the world and is notorious for transmitting dengue and yellow fever. Although some 2.5 billion people live in dengue-endemic areas, and an estimated 50 million are infected every year, there is still no effective vaccine against the disease and the prevention of viral transmission depends entirely on controlling the mosquito vector or interrupting human–vector contact. Traditional measures to control *A. aegypti* involve application of a larvicidal organophosphate, such as Temephos, to larval habitats. However, the constant use of such compounds has resulted in the appearance of resistant populations of *A. aegypti* (Rodríguez et al., 2002; Garcia et al., 2009; Melo-Santos et al., 2010). A recent study

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has shown that the parasitic microbe *Wolbachia pipientis* can affect reproduction and longevity of *A. aegypti* (Hoffmann et al., 2011), but this gram-negative proteobacterium also infects non-target species and may thus disrupt the ecological equilibrium. There remains an urgent need for the discovery of different larvicidal products for application against the mosquito vector.

In a similar manner, the search for alternative agents to control microorganisms has gained importance recently by virtue of the reduced susceptibility that many important pathogens have shown to conventional antibiotics. In this context, essential oils, many of which display potent antimicrobial properties, could be employed in the control of bacteria and other microorganisms (Carson and Hammer, 2011).

In the present paper, we report on the constituents of the essential oils obtained by hydrodistillation of the floral bracts of red and pink variants of *A. purpurata*, and on the larvicidal, oviposition deterrent and antibacterial properties of the oils, aqueous extracts and hydrolates derived therefrom.

2. Methods

2.1. Plant material

Fresh inflorescences of a red cultivar (Red Ginger) and of a pink variant (Eileen McDonald) of *A. purpurata* were obtained from a commercial grower (Estação Tropical, Paulista, PE, Brazil). The plant material was authenticated by Marlene Barbosa (Herbário UFP Geraldo Mariz, Universidade Federal de Pernambuco), and a voucher specimen has been deposited at the herbarium with the identification number 53376. The plants (equal proportions of red and pink varieties) employed in the experiments were cultivated in a 50,000 m^2 area under full sunlight, at 22–35 °C and 60–80% humidity. Flowers were harvested in the early morning (0500 h) when two-thirds of the inflorescences were still closed. The exact day of collection varied according to the intensity of light during growth, but occurred approximately 10 days after the emergence of inflorescences. Cut flowers were washed thoroughly under tap water in order to remove insects *etc.*, and placed in boxes for transportation. In the laboratory, the flowers were immersed in water for 3 h and subsequently maintained in a refrigerator for 1–2 days until required for extraction.

2.2. Preparation of essential oils and aqueous extracts

Floral bracts and axes (*ca.* 800 g) were comminuted in a blender and hydrodistilled with 3 L of distilled water for 3 h in a Clevenger-type apparatus. The essential oil layer was separated, dried over anhydrous sodium sulphate and stored in a hermetically sealed amber-glass vial at −5 °C until required for assay. The yield of oil was reported as the quotient of the mass of oil collected and the fresh weight of plant material extracted (Autran et al., 2009). The aqueous extract (the aqueous solution remaining following extraction of the essential oil) was cooled, filtered and stored at −5 °C until required for assay. The hydrolate was also collected for assay of larvicidal activity.

2.3. Hyphenated gas chromatographic (GC) analyses

Analyses were carried on a Hewlett-Packard 5890 Series II gas chromatograph equipped with a non-polar DB-5 (J & W Scientific) fused silica capillary column (30 m × 0.25 mm i.d.; film thickness 0.25 mm) and a flame ionisation detector (FID). The carrier gas was hydrogen supplied at a flow rate of 1 L/min and 30 psi inlet pressure. The injector and detector were maintained at 250 °C, and the oven temperature was programmed from 50 °C to 250 °C at 3 °C/min. The sample (1 μL; containing oil diluted 1:50, v/v with hexane) was introduced onto the column with the injector in split-mode (1:50 split ratio). Analyses were conducted in triplicate, and the data from one chromatogram are compiled in Table 1 as representative of the analytical results obtained. The relative amount (uncorrected) of each component was estimated from the corresponding peak area expressed as a percentage of the total peak area on the chromatogram.

GC–mass spectrometric (MS) analyses were carried out using a Shimadzu model QP 5050 quadrupole instrument equipped with a non-polar DB-5 (J & W Scientific) fused silica capillary column (30 m × 0.25 mm i.d.; film thickness 0.25 mm). The carrier gas was helium supplied at a flow rate of 1 L/min. The injector and detector were maintained at 250 °C and 280 °C, respectively, and the oven temperature was programmed a 40 °C, held for 2 min, increased to 220 °C at 4 °C/min, then increased to 280 °C at 20 °C/min and finally held for 10 min. The sample (1 μL; containing 3 mg/mL of essential oil in hexane) was introduced onto the column with the injector in splitless mode. The ionisation potential was 70 eV, while the scan range was from m/z 40 to 550 with a scan rate of 0.5 scans/s (Autran et al., 2009).

2.4. Identification of oil constituents

Retention indices (RI) were determined for the separated components of the essential oils by co-injection of samples with a mixture of C_8–C_{30} linear hydrocarbons and subsequent application of the Van den Dool and Kratz (1963) equation. Constituents were initially identified by comparison of RI values with those published in the literature. Identities were confirmed by matching the acquired mass spectra with those stored in the Wiley/NBS mass spectral library of the GC–MS system and with other published data (Adams, 2007).

2.5. Maintenance of A. aegypti

A colony of *A. aegypti* was maintained in the laboratory at 28 ± 1 °C, 70 ± 5% relative humidity and a photoperiod of 14L:10D. Adult mosquitoes were maintained on a 10% sucrose solution, while females were blood-fed on pigeons. Larvae were reared in plastic dishes and fed on a diet of minced commercial cat food (Whiskas®).

2.6. Assay of larvicidal activity

Stock solutions containing 100 ppm of the essential oils were prepared by dissolving 5.00 mg of the oil in 0.7 mL of ethanol and completing to 50 mL with distilled water. These stock solutions were used to prepare test solutions with concentrations in the range 10–100 ppm for use in the larvicidal bioassays. The concentrations of aqueous extracts obtained from hydrodistillation were considered to represent the 100% levels. These aqueous extracts were diluted with distilled water to provide final concentrations of 5, 10, 15, 20, 25 and 30% by volume for use in the larvicidal bioassays. The hydrolates were assayed undiluted. α-Pinene (purity ≥ 99%), β-pinene (purity ≥ 99%) and β-caryophyllene (purity ≥ 98.5%) were obtained from Sigma–Aldrich and included in the larvicidal assays. Stock solutions (400 ppm) were prepared by dissolving 20.00 mg of the pure compound in 0.7 mL of dimethylsulphoxide (DMSO) and completing to 50 mL with distilled water.

Larvicidal activities were evaluated using a modified version (Navarro et al., 2003) of the method recommended by the World Health Organization (2005). Early 4th instar *A. aegypti* were transferred to disposable cups (20 larvae per cup) containing test samples in a range of concentrations obtained (where appropriate) by dilution of stock solutions with distilled water. Four replicate assays were carried out for every test sample at each concentration,

Table 1
Identification of constituents of the essential oils of red and pink variants of *Alpinia purpurata*.

Number	Identity[a]	RI determined[b]	RI literature[c]	Content (as % of total oil)	
				Red variant	Pink variant
1	α-Pinene	933	932	6.28	13.86
2	Camphene	947	947	2.76	7.05
3	β-Pinene	974	974	13.93	26.56
4	Myrcene	998	988	0.26	1.37
5	α-Terpinene	1012	1014	–[d]	0.12
6	*p*-Cymene	1019	1020	–	0.17
7	Limonene	1026	1024	1.68	5.74
8	*trans*-Ocimene	1044	1044	–	0.09
9	γ-Terpinene	1055	1054	–	0.36
10	Terpinolene	1086	1086	0.36	0.1
11	Linalool	1095	1095	9.64	0.53
12	n-Nonanal	1098	1100	–	1.28
13	Endo-fenchol	1111	1114	0.38	–
14	Camphor	1138	1141	0.87	–
15	Camphene hydrate	1144	1145	0.56	–
16	Borneol	1163	1165	1.53	–
17	Terpinen-4-ol	1174	1174	0.86	–
18	α-Terpineol	1186	1186	7.16	0.1
19	Fenchyl acetate	1216	1218	–	0.48
20	Endo-fenchyl acetate	1218	1218	0.37	–
21	Bornyl acetate	1283	1287	3.21	2.54
22	β-Elemene	1389	1389	0.27	0.18
23	*α*-Caryophyllene	1404	1408	–	0.09
24	β-Caryophyllene	1423	1417	18.26	15.58
25	α-Humulene	1454	1452	2.23	1.75
26	(*E*)-4,10-epoxy-Amorphane	1476	1478	–	0.15
27	β-Chamigrene	1485	1476	4.37	–
28	α-Selinene	1485	1498	–	2.27
29	β-Selinene	1488	1489	2.89	1.74
30	Valencene	1497	1496	5.22	2.97
31	7-epi-α-Selinene	1521	1520	8.26	4.68
32	Nerolidol	1561	1561	1.27	0.7
33	Caryolan-8-ol	1572	1571	0.1	–
34	Caryophyllene oxide	1581	1582	1.83	1.44
35	Fokienol	1594	1596	0.13	–
36	Guaiol	1599	1600	0.08	–
37	Humulene epoxide II	1609	1608	0.16	0.11
38	γ-Eudesmol	1634	1630	0.43	–
39	Caryophylla-4(12),8(13)-dien-5β-ol	1638	1639	0.55	–
40	β-Eudesmol	1653	1649	0.23	–
41	Neointermedeol	1657	1658	0.13	0.52
42	Intermedeol	1662	1665	1.43	–
Monoterpene hydrocarbons				25.27	56.42
Oxygenated monoterpenes				24.58	3.65
Sesquiterpene hydrocarbons				41.50	29.26
Oxygenated sesquiterpenes				6.34	2.92
Total				97.69	93.53

[a] Constituents listed in order of elution on a non-polar DB-5 column.
[b] Retention indices (RI) calculated from retention times in relation to those of a series of C_8–C_{30} *n*-alkanes on a 30 m DB-5 capillary column.
[c] Values taken from Adams (2007).
[d] Not detected.

and negative controls (distilled water containing the same amount of co-solvent, where appropriate, as the test sample) were included in each assay. Larval mortality, assessed as lack of response to stimulus or larvae not rising to the surface, was determined after 24 and 48 h, and median lethal concentration (LC_{50}) values were calculated by Probit analysis using StatPlus2008 software.

2.7. Assay of oviposition deterrent activity

Test samples containing 100 ppm of essential oils were prepared by dissolving 20.00 mg of oil in 1.5 mL of ethanol and completing to 200 mL with distilled water. Control solutions for these assays were prepared by diluting 1.5 mL of ethanol to a final volume of 200 mL with distilled water. Assays with aqueous extracts were performed using 20% (v/v) solutions and with distilled water as control.

Gravid *A. aegypti* females were transferred to cages measuring 33 cm × 21 cm × 30 cm with 10 individuals per cage. Two disposable cups were placed at diagonally opposite corners of the cage; one of the cups was filled with 25 mL of the test solution and the other with 25 mL of the appropriate control. Filter paper was placed on the internal surface of each cup to provide a support for oviposition. Cages were maintained at 28 ± 1 °C and 70 ± 5% relative humidity, for 16 h in the dark, after which the oviposition response was determined by counting the numbers of eggs laid on the filter papers. Each experiment was replicated at least eight times, and negative controls (distilled water containing the same amount of co-solvent, where appropriate, as the test sample) were included in the assays. Mean values obtained for each of the treatments were compared using Student's *t*-test at an α level of 0.05 (Navarro et al., 2003).

2.8. Assay of antibacterial activity

The essential oil of *A. purpurata* cultivar Red Ginger was assayed against gram-positive *Staphylococcus aureus* (non-resistant and oxacillin-resistant strains) and *S. epidermis*, and gram-negative *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella* sp., *Shigella* sp., *Proteus* sp. and *Klebsiella* sp. All bacterial strains were isolated from hospitals or clinical laboratories except for *S. aureus* ATCC6538, *S. aureus* UFPEDA02, *E. coli* ATCC25922 and *P. aeruginosa* ATCC27853, which were obtained from the collection held by the Departamento de Antibióticos at the Universidade Federal de Pernambuco. Sets of Petri dishes were prepared with Muller-Hinton agar medium to which appropriate volumes of a solution containing 20,000 μg/mL of the essential oil in DMSO had been added. Microorganisms were streaked across the surface of the media and the plates were incubated at 35 °C for 24 h. The minimum inhibitory concentration (MIC; Agência Nacional de Vigilância Sanitária, 2005) was assessed as the lowest concentration of essential oil able to inhibit the visible growth of the bacterium. The experiments were conducted in duplicate and the concentrations of the samples were fixed (10, 30, 125, 225, 500 and 1000 ppm).

3. Results and discussion

The yields of essential oils obtained by hydrodistillation of floral bracts of the red and pink variants of *A. purpurata* were 0.052 and 0.047%, respectively. A total of 42 mono- and sesqui-terpenes were identified by GC–MS analysis (Table 1), of which 19 were present in the oils of both variants, 14 were detected only in the red cultivar, and 9 were unique to the pink variety. Although the major constituents of oils of both variants were β-pinene and β-caryophyllene, sesquiterpene hydrocarbons formed the predominant group in the red cultivar, while monoterpene hydrocarbons were the principal components of the pink variant. Interestingly, while monoterpene hydrocarbons and oxygenated monoterpenes were equally represented in the red cultivar, oxygenated C_{10} compounds were not abundant in the pink form. The differences in essential oil composition might indicate that the two cultivated forms of *A. purpurata* constitute dissimilar chemotypes. According to Llamas (2003), it is not clear if the pink form is a cultivar or a natural variant.

The composition of the essential oil of floral bracts of *A. purpurata* cultivar Red Ginger reported in the present study is similar to that previously established by Zoghbi et al. (1999). However, essential oils are more commonly isolated from leaves, seeds or rhizomes of *Alpinia* spp. rather than from the bracts. In this context, Victório et al. (2010) recorded a different profile for the essential oil from leaves of *A. purpurata* in which the main constituents, β-pinene (34.7%) and α-pinene (11.8%), co-occurred with components not detected in the floral oil, namely, *trans*-β-guaiene (6.0%), *p*-cymen-7-ol (4.8%), neryl-acetone (3.3%) and the diterpene 3-α-hydroxy-manool (3.2%).

A number of studies are available concerning the essential oils derived from other *Alpinia* species. Thus, the oil from fresh leaves of *A. zerumbet* was shown to contain terpinen-4-ol (28.09%), 1,8-cineole (15.02%), γ-terpinene (13.71%), p-ocimene (7.35%) and sabinene (7.09%) as major constituents (Lahlou et al., 2003), while that from fresh leaves of *A. speciosa* was rich in camphor (31.6%), with sabinene (9.4%), γ-terpinene (8.0%) and 1,8-cineole (5.6%) as other important constituents. The essential oil from the seeds of the latter species also contained camphor (19.3%) and sabinene (15.1%) as major constituents (Ho, 2010). With respect to essential oils derived from rhizome tissue, that from *Alpinia latilabris* comprised methyl-(*E*)-cinnamate as the sole major component representing 89.5% of the total oil, while the rhizome oil of *Alpinia conchigera* contained β-bisabolene (28.9%), 1,8-cineole (15.3%), β-caryophyllene (10.0%) and β-pinene (9.5%) as main components (Wong et al., 2005).

Table 2
Activities against fourth stage larvae of *Aedes aegypti* of the hydrodistillation derivatives obtained from red and pink variants of *Alpinia purpurata*.

Extract	LC_{50} (confidence interval)[a]	Standard error
Hydrolates		
Red and pink variants	Not active	
Aqueous extracts		
Red variant	18.3%[b] (15.5–21.1)	±1.1
Pink variant	12.6%[b] (10.5–14.6)	±1.4
Essential oils		
Red variant	80.7 ppm (75.1–86.3)	±2.8
Pink variant	71.5 ppm (64.6–78.4)	±3.5

[a] Median lethal concentration values (LC_{50}) and 95% confidence intervals are means of four replicate determinations.
[b] Percentage concentration (v/v) of aqueous extract.

Following a study of essential oils obtained from flowers, leaves and rhizomes of *A. calcarata*, *A. speciosa*, *A. allughas* and two chemotypes of *A. galanga*, Padalia et al. (2010) concluded that monoterpenes were generally present in major proportions (59.5–90.0%), and that most of the oils contained pinene or caryophyllene. In essential oils from leaves and flowers, the most common major constituents were found to be 1,8 cineole, α-terpineol, methyl-(*E*)-cinnamate, camphor, terpinen-4-ol, and α- and β-pinene.

The essential oils and aqueous extracts derived from red and pink variants of *A. purpurata* showed significant activities against early 4th instar *A. aegypti* (Table 2). In contrast, hydrolates from both variants were innocuous to the larvae. No mortalities were observed in any of the control assays, indicating that the amounts of organic solvents used to solubilise the samples were less than those required to cause damage to the larvae, a finding in accord with that of Kramer et al. (1983). No significant differences were detected between the LC_{50} values recorded for the essential oils obtained from the red (80.7 ± 2.8 ppm) and the pink (71.5 ± 3.5 ppm) variants of *A. purpurata* and both were <100 ppm, the value considered to be the upper limit for preparations described as active (Cheng et al., 2003). The aqueous extracts of the red and pink variants showed 100% larval mortality when diluted 50% with distilled water, and their LC_{50} values (18.3 and 12.6%, respectively) were not significantly different. Larvae that had been submitted to treatment with aqueous extracts showed prominent morphological alterations, with modified colouration (darkening of thoracic and dorsal regions) and extrusion of gut material (possibly peritrophic membrane) from the mouth or anus. Further investigations relating to this effect are currently underway in our laboratories. Interestingly, aqueous extracts are usually disregarded as by-products of hydrodistillation but, in the case of *A. purpurata*, such material may be of value in the control of *A. aegypti* larvae.

Numerous studies have demonstrated that essential oils are able to induce mortality in *A. aegypti* larvae (Silva et al., 2008; Cheng et al., 2009), but the present study is the first to demonstrate the larvicidal activity of *A. purpurata*. With respect to other species of *Alpinia*, Cavalcanti et al. (2004) reported that essential oil from the leaves of *A. zerumbet* presented an LC_{50} of 313 ppm against 3rd instar *A. aegypti*, while the oil from fresh leaves and seeds of *A. speciosa* showed LC_{50} values of 32 and 87 ppm, respectively, against 4th instar *A. aegypti* (Ho, 2010).

The major components identified in the essential oils of *A. purpurata*, *i.e.* α- and β-pinene and β-caryophyllene, were assayed individually against 4th instar *A. aegypti* (Table 3). The larvicidal activities of the pure compounds were relatively low in comparison with those of the essential oils tested, and significant levels of mortality could only be detected at concentrations in excess of 150 ppm.

Table 3
Activities against fourth stage larvae of *Aedes aegypti* of β-pinene, α-pinene and β-caryophyllene.

Concentration of terpene (ppm)	Mortality (%) ± standard error		
	α-Pinene	β-Pinene	β-Caryophyllene
150	12 ± 0.3	35 ± 0.6	3.3 ± 0.2
200	27 ± 0.6	40 ± 1.1	3.3 ± 0.2
250	33 ± 0.6	65 ± 0.6	3.3 ± 0.2
300	45 ± 0.0	78 ± 0.6	5.0 ± 0.8
400	67 ± 1.9	90 ± 1.0	3.3 ± 0.2

Such findings are in accordance with the previously reported high LC_{50} values of *ca.* 500 ppm for α- and β-pinene (Waliwitiya et al., 2008) and 1202 ppm for β-caryophyllene (Silva et al., 2008) assayed against 4th instar *A. aegypti*, and indicate that these terpene hydrocarbons are not the main compounds responsible for the larvicidal activities of the essential oils assayed. Moreover, it is important to note that larvicidal assays employing high concentrations of pure compounds can be affected by solubility problems since the amount of co-solvent used in preparing stock solutions of samples is limited in order to avoid interference in larvae mortality. When the test compound is completely soluble in the bioassay solution, larval mortality can be promoted by ingestion or skin adsorption (Bakkali et al., 2008). If, however, the permitted percentage of co-solvent is insufficient to solubilise completely the test compound, then droplets of undissolved sample will be distributed within the aqueous solution. In this case, a thin film of insoluble compound may form on the surface of the aqueous solution and this can affect larval respiration leading, eventually, to mortality (Bakkali et al., 2008). Since thin films are not formed homogeneously over the entire surface of the aqueous solution, larval mortality caused by this process would not exhibit a linear response with respect to compound concentration.

Considering the minor components present in the essential oil of *A. purpurata*, some relevant larvicidal activities can be found in the literature, including LC_{50} values of 125 ppm for caryophyllene oxide (Silva et al., 2008), 19.2 ppm for *p*-cymene, 28.4 ppm for terpinolene, 14.7 ppm for α-terpinene, >50 ppm for α-terpineol, >50 ppm for terpinen-4-ol (Cheng et al., 2009), between 30.7 and 95 ppm for γ-terpinene, and between 18.1 and 37 ppm for limonene (Silva et al., 2008; Cheng et al., 2009; Santos et al., 2011). Of the compounds cited above, limonene, terpinolene and caryophyllene oxide were present in both varieties. Other minor compounds as bornyl acetate, β-elemene, α-humulene, nerolidol and β-selinene were present in similar concentrations in both varieties. Since the data provided by the larvicidal bioassays were similar as well, we suggest that these compounds could be responsible for the observed activities. Any or all of these components might modulate the biological activity of the essential oil of *A. purpurata*. Moreover, since essential oils are complex mixtures, their overall effects are often mediated by interactions between the different constituents and with the biological system (Bakkali et al., 2008).

Essential oils (at 100 ppm) and aqueous extracts (at 20%, v/v) derived from the red and pink variants of *A. purpurata* showed significant ($p < 0.05$) oviposition deterrent effects, in that the numbers of eggs laid on filter papers in cups containing test solution were significantly lower than those in the controls (Fig. 1). No oviposition assays were performed with the hydrolates since they were found to be inactive in the larvicidal tests. The oviposition results reported here are similar to those obtained for *A. galanga* (Tawatsin et al., 2006). In the present study, the essential oils of *A. purpurata* promoted significant larvicidal effects against *A. aegypti* in the concentration range 71.5–80.7 ppm and oviposition deterrent effects at 100 ppm, while the aqueous extracts were similarly active.

Table 4
Activities against gram-positive and gram-negative bacteria of the essential oil obtained from *Alpinia purpurata* cultivar Red Ginger.

Bacterium	Minimum inhibitory concentration (μg/mL)
Staphylococcus aureus ATCC6538	<10
S. aureus UFPEDA 02	<10
S. aureus ORSA A64	30
S. aureus ORSA A5555	<10
S. aureus ORSA A5409	1000
S. aureus ORSA A71	500
S. aureus ORSA A5107	<10
S. aureus ORSA A57	<10
S. aureus ORSA A5563	<10
S. aureus ORSA A68	<10
S. aureus ORSA A5201	500
S. aureus ORSA A55	1000
S. aureus ORSA A5660	125
S. aureus ORSA A179	125
S. epidermis	<10
Escherichia coli ATCC25922	1000
E. coli (resistant)	1000
Salmonella sp.	1000
Shigella sp.	1000
Pseudomonas aeruginosa ATCC27853	1000
Klebsiella sp.	1000
Proteus sp.	>1000

In related studies, a 20 mg/L solution of the essential oil of *Rosmarinus officinalis* was reported to induce a strong oviposition deterrence response against *A. aegypti* over a 5 day bioassay (Waliwitiya et al., 2008), while the essential oils from stems, leaves and inflorescences of *Piper marginatum* showed oviposition deterrent effects at concentrations of 100 ppm with 40% inhibition in comparison with controls (Autran et al., 2009).

The choice of oviposition sites by gravid female mosquitoes is guided by several factors. Initially, visual and olfactory cues are employed to find potential sites, following which the suitability of the location is verified with respect to chemical and physical factors by appropriate receptors distributed along the body of the mosquito. Clearly, when oviposition deterrents are detected, few, if any, eggs are laid at the site (Bentley and Day, 1989).

The MIC values obtained from the antibacterial assays performed using the essential oil of *A. purpurata* cultivar Red Ginger are shown in Table 4. It is well known that most essential oils exhibit some level of antibacterial activity but, according to Carson and Hammer (2011), only those that kill or inhibit bacterial growth at concentrations below 1% (10,000 ppm) should be considered active. On this basis, the results presented here reveal that *A. purpurata* oil may be regarded as active against most of the bacterial strains tested with the exception of *Proteus* sp., which grew even at the highest concentration of oil assayed (1000 μg/mL). The lowest MIC values were recorded for the gram-positive species, indeed some *S. aureus* ORSA strains were highly susceptible to *A. purpurata* oil with MIC values < 10 μg/mL. In contrast, the MIC values for gram-negative species were typically around 1000 μg/mL.

Unfortunately, only essential oil from the red form of *A. purpurata* could be assayed for antibacterial activity since the availability of material from the pink variant was limited. The reason for this is that, while both red and pink variants are harvested on a large scale throughout the year in the state of Pernambuco, the production of pink flowers is significantly lower than that of the red.

The present study is the first to report the antibacterial activity of the essential oil of *A. purpurata*, although other species of the genus are known to be inhibitory against microorganisms. Thus, the essential oil from *A. conchigera* showed weak inhibition against *P. aeruginosa*, *S. aureus* and *S. epidermis* (Ibrahim et al., 2009), that from *A. galanga* inhibited the growth of gram-positive and gram-negative bacteria (Yusoff et al., 2011), while *A. speciosa* oil

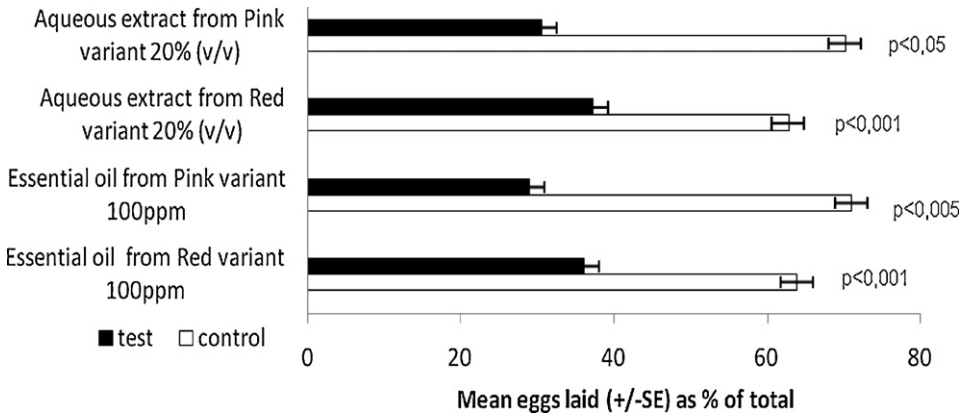

Fig. 1. Oviposition responses of gravid *Aedes aegypti* to aqueous extracts and essential oils of *Alpinia purpurata*. Each assay involved 10 gravid female mosquitoes and assays were replicated 8 times. The values represent mean percentages (±SE) of the total eggs laid in response to the treatment.

presented antibacterial activity against the gram-positive bacteria *Micrococcus luteus*, *Streptococcus mutans*, *Bacillus subtilis* and *S. aureus*, and against the gram-negative bacteria *Salmonella typhi* and *P. aeruginosa* (Indrayan et al., 2010). It is reported that MIC values of the essential oil from rhizomes of *A. pahangensis* are as low as those of the reference antibiotic oxacillin when assayed against five strains of *S. aureus* (Awang et al., 2011).

4. Conclusion

Flowers of *A. purpurata* that are not of sufficiently high quality for direct commercialisation can be hydrodistilled to provide an essential oil and an aqueous extract, both of which could be exploited as larval insecticides for application in the fight against the spread of *A. aegypti*. Moreover, the floral oil from *A. purpurata* exhibits great potential as an antibacterial agent that could be employed in pharmacological formulations.

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