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Original Article

Dendranthema grandiflorum, a hybrid ornamental plant, is a source of larvicidal compounds against *Aedes aegypti* larvae

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ABSTRACT

In hybrid cultivated form, *Dendranthema grandiflorum* (Ramat.) Kitam., Asteraceae, flowers (*Chrysanthemum morifolium* Ramat.) were utilized in the production of extracts, which were analyzed for larvicidal activity against *Aedes aegypti* third instar larvae. Methanol and dichloromethane extracts showed LC_{50} values of 5.02 and 5.93 ppm, respectively. Using GC–MS, phytochemical analyses of the dichloromethane extract showed the presence of triterpenoids and fatty acids, while flavonoids and caffeoylquinic acids were shown to occur in the methanol extract by ESI Fourier Transform Ion Cyclotron Resonance Mass Spectrometry (ESI-FT-ICR-MS). Triterpenoids and fatty acids are well known insecticidal compounds. From this study, it can be concluded that *D. grandiflorum* grown for floriculture, as an agribusiness, can have additional applications as raw material for the production of insecticidal products.

Introduction

Known for over 2000 years, the chrysanthemum is an ornamental plant native to China. It belongs to the genus *Dendranthema*, Asteraceae, and numerous cultivars have been produced (Kim et al., 2015). The cut chrysanthemum is one of the most commercially successful flowers by the diversity of its inflorescence (Brackmann et al., 2005). Floriculture is a representative activity in Brazilian agribusiness and, as such, it has made an outstanding economic and technological contribution. In particular, the chrysanthemum culture (*Dendranthema grandiflorum* (Ramat.) Kitam.) has great importance in Brazilian floriculture, and its cultivation has increased throughout the country (Polanczyk et al., 2008). Climate is an important factor that favors the expansion of floriculture, as an agribusiness, in Brazil. Specifically, it allows for the cultivation of temperate and tropical flowers with low cost and high production all year long (Da Silva Junior et al., 2011).

Dengue is a major public health problem in Brazil. The transmission of the dengue virus to humans occurs through the bite of the mosquito *Aedes aegypti*. The disease can be temporarily disabling, but in its hemorrhagic form, it can result in death. Although public policies exist for mosquito control, resistance to conventional insecticides is concerning to health authorities (Liu, 2015). Therefore, this paper describes a natural product, extracts of *D. grandiflorum*, which are obtained from the cultivar "yellow sheena", as a source of compounds with larvicidal activity against *A. aegypti* third instar larvae. The studied plant is organically cultivated in the State of Rio de Janeiro, Nova Friburgo City. In view of its larvicidal potential, the cut flower should also support the establishment of floriculture as an important agribusiness in Brazil.

Materials and methods

Plant material

Dendranthema grandiflorum (Ramat.) Kitam., Asteraceae, yellow sheena, a hybrid under organic cultivation, was obtained from farmers in Nova Friburgo, State of Rio de Janeiro, in September 2013 and botanically identified by Dr. Mariana Machado Saavedra from

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the Rio de Janeiro Botanical Garden (voucher specimen deposited at the herbarium Prof. Jorge Pedro Pereira Carauta under number HUNI 3531).

Preparation of extracts

The flowers were dried in an oven at 50 °C for 7 days (223 g). After that, they were submitted to methanol extraction over a period of 15 days. The extract was filtered and the solvent removed by rotative evaporation under vacuum to obtain the first product, a dry MeOH extract (80 g). Seventy grams were suspended in water, and after solvent removal, the suspension was partitioned with dichloromethane to yield the second product, a dry CH_2Cl_2 extract (29 g).

Phytochemical analysis

Electron Impact Ionization/Mass Spectrometry (EI/MS) was obtained using a Shimadzu QP5050 GC–MS on a DB-5 column (30 m × 0.25 mm and film thickness of 0.25 μm, J&W Scientific) with an ionizing energy of 70 eV. Programming of the oven temperature started at 80 °C, which was maintained for 2 min. Then, the temperature was raised to 260 °C at a rate of 15 °C/min and again increased to 320 °C at 5 °C/min. An isotherm during 10 min was performed at this final temperature. The temperatures of injector and ion source were kept at 250 °C and 280 °C, respectively. Helium was used as the carrier gas with a constant flow of 1.1 ml min^{-1}. One microliter of the sample was injected with a split ratio of 70:1. Mass spectra of extract compounds were compared with the NIST Mass Spectral Database (MS Search 2.0) and with literature data (Assimoupoulou and Papageorgiou, 2005).

The methanolic extract of *D. grandiflorum* was analyzed in a mass spectrometer (Model 9.4 T Solarix, Bruker Daltonics, Bremen, Germany), which was set to operate in negative ion mode, ESI(-), over a mass range of *m/z* 200–1300. The parameters of the ESI(-) source were as follows: nebulizer gas pressure of 0.5–1.0 bar, capillary voltage of 3–3.5 kV, and transfer capillary temperature of 250 °C. The mass spectrum was processed using the Compass Data Analysis software package (Bruker Daltonics, Bremen, Germany). A resolving power, $m/\Delta m_{50\%} \cong 500{,}000$, in which $\Delta m_{50\%}$ is the full peak width at half-maximum peak height of $m/z \cong 400$ and a mass accuracy of <1 ppm, provided the unambiguous molecular formula assignments for singly charged molecular ions. Elemental compositions of the compounds were determined by measuring the *m/z* values. The unsaturation level of each molecule could be deduced directly from its double bond equivalent (DBE), following the equation DBE = c − h/2 + n/2 + 1, where c, h, and n are the numbers of carbon, hydrogen, and nitrogen atoms, respectively (Ferreira et al., 2014; Costa et al., 2014; De Sá et al., 2015; Nascimento et al., 2015). Molecular formula, measured *m/z* values, DBE, and mass error are shown in Table 1. Tandem mass spectrometry (MS^2) experiments were also performed on a quadrupole analyzer coupled to the FT-ICR mass spectrometer, and quadrupole Fourier transform ion cyclotron resonance mass spectrometry (Q-FT-ICR MS) was performed for ions of *m/z* 353, 431, 447, 449 and 515. The fragments produced (*m/z* values) from ESI(-)-MS/MS spectra are shown in Table 3.

Larvicidal activity

A. aegypti larvae that originated from the NPPN strain were reared in the laboratory under controlled photoperiod (12 h light and 12 h dark) at 27 °C and 80 ± 10% rel. humidity in trays filled with dechlorinated tap water and canine food.

Larvicidal activity was conducted following the method adapted from WHO (1970). For each treatment and control, five larvae of third stage instars, or early fourth stage instars, were transferred into 20 ml glasses in 14.9 ml of distilled water. The solutions of crude extracts, or fractions, were prepared in ethanol and added (0.1 ml) to the treatment glasses with a pipette to give the final assay concentrations. Controls received aqueous solution with 0.1 ml of ethanol only. After 24 h, the number of dead larvae in each glass was counted. All treatments were replicated three times. The 50% lethal concentration (LC_{50}) was calculated with a 95% confidence limit by Probit analysis.

Results and discussion

Phytochemical analysis

As shown in Fig. 1, methanol extract of *D. grandiflorum* by ESI(-)FT-ICR MS yielded 11 compounds identified as fatty acids, phenylpropanoids and flavonoids (Table 1). All compounds were detected as deprotonated molecules, *i.e.*, adduct ion, as represented by $[M-H]^-$ ion, formed by the interaction of a molecule with a proton, or hydrogen (Table 1). Among fatty acids, palmitic acid, *m/z* 255.2330, was the major compound found in the crude extract. Phenylpropanoids, such as monocaffeoylquinic acids,

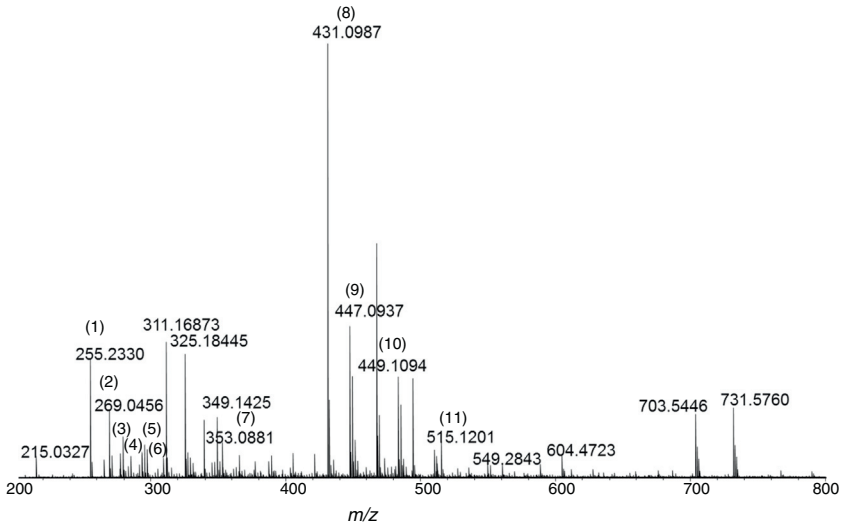

Fig. 1. ESI(-)FT-ICR mass spectrum of MeOH extract of *Dendranthema grandiflorum* flowers.

Table 1
Fatty acids, flavonoids and caffeic acid derivatives of methanol extract of *Dendranthema grandiflorum*, as identified from ESI(-)-FT-ICR MS.

Identification	$m/z_{measured}$	Identified compounds	$[M-H]^-$	Fragments MS/MS	DBE	Error (ppm)
1	255.2330	Palmitic acid	$C_{16}H_{31}O_2$	–	1	0.05
2	269.0456	Apigenin	$C_{15}H_9O_5$	–	11	0.26
3	279.2330	Linoleic acid	$C_{18}H_{31}O_2$	–	3	0.32
4	285.0406	Luteolin	$C_{15}H_9O_6$	–	11	0.31
5	293.2123	Linolenic acid methyl ester	$C_{18}H_{29}O_3$	–	4	0.30
6	295.2280	Linoleic acid methyl ester	$C_{18}H_{31}O_3$	–	3	0.45
7	353.0881	Monocaffeoylquinic acid	$C_{16}H_{17}O_9$	191	8	0.77
8	431.0987	Apigenin 7-*O*-glucoside	$C_{21}H_{19}O_{10}$	269	12	0.65
9	447.0937	Luteolin 7-*O*-glucoside	$C_{21}H_{19}O_{11}$	285	12	0.90
10	449.1094	Eriodictyol 7-*O*-glucoside	$C_{21}H_{19}O_{11}$	431, 413, 387, 311, and 287	11	1.01
11	515.1201	Dicaffeoylquinic acid	$C_{25}H_{23}O_{12}$	353	14	1.18

Table 2
Composition of fatty acids, steroids and triterpenoids in the dichloromethane partition of *Dendranthema grandiflorum*.

Retention time – RT (min)	Major fragments from MS data	Identified compounds	Relative concentration (%)
12.95	270 (M^+), 227, 143, 99, 87, 74 (100%), 55	Methyl palmitate	2.98
13.20	256 (M^+), 213, 185, 171, 157, 143, 129, 115, 83, 73, 57 (100%)	Palmitic acid	3.97
13.40	284 (M^+), 256, 241, 227, 213, 157, 101, 88 (100%), 83, 73, 55	Ethyl palmitate	0.23
14.15	294 (M^+), 263, 95, 81, 67 (100%), 55	Linoleic acid methyl ester	0.15
14.20	292 (M^+), 264, 149, 135, 122, 108, 93, 79 (100%), 67, 55	Linolenic acid methyl ester	0.17
14.36	298 (M^+), 255, 199, 143, 87, 74 (100%), 55	Methyl stearate	0.12
Total relative concentration of fatty acids			8.42
32.75	412 (M^+), 55 (100%)	Stigmasterol	3.68
33.79	414 (M^+), 55 (100%)	Sitosterol	13.47
34.45	426 (M^+), 218 (100%), 203 (49%), 189	β-Amyrin	13.53
35.20	426 (M^+), 218 (100%), 203 (18%), 189	α-Amyrin	22.03
35.79	468 (M^+), 218 (100%), 207 (61%), 203 (40%), 189 (20%)	β-Amyrin acetate	1.68
36.50	468 (M^+), 218 (100%), 207 (67%), 203 (21%), 189 (40%)	α-Amyrin acetate	4.78
36.68	426 (M^+), 207, 189 (100%), 175, 147, 135, 121, 109, 95, 81, 68, 55	Lupeol	5.06
Total relative concentration of triterpenoids and steroids			64.23%

m/z 353.0878, and dicaffeoylquinic acids, *m/z* 515.1201, were detected as minor compounds. Among the flavonoids identified, apigenin 7-*O*-glucoside, *m/z* 431.0987, eriodictyol 7-*O*-glucoside, *m/z* 449.1093, and luteolin 7-*O*-glucoside, *m/z* 447.0933, were the main compounds of the MeOH extract. Purification and NMR identification of the flavonoids were described by Spindola (2015), and all the compounds were described to occur in *D. grandiflorum* (Lin and Harnly, 2010; Uehara et al., 2012). Table 1 shows the identified compounds for fatty and caffeic acids, as well as flavonoids of *D. grandiflorum* methanol extract, as identified from ESI(-)-FT-ICR MS data.

GC–MS analyses of CH_2Cl_2 extract shows abundant peaks in two different regions in the chromatogram: (i) from 6 to 18 min and (ii) from 30 to 38 min. In general, fourteen compounds were identified, and their proposed structures, molecular ions and fragments, retention time, and abundance relative values are summarized in Table 2.

In the first region (approximately between 13.0 and 15.0 min), the major fatty acids were as follows: methyl palmitate, ethyl palmitate, palmitic acid, stearic acid, ethyl stearate, linoleic acid methyl ester and linolenic acid methyl ester (Table 2). However, in the second region (32–38 min), steroids (sitosterol and stigmasterol) and triterpenoids (β-amyrin, α-amyrin, β-amyrin acetate, α-amyrin acetate and lupeol) can be seen as less volatile substances of the chromatogram. They were previously identified in *Chrysanthemum morifolium* (Takahashi and Sato, 1979). The relative abundances of the two main classes of identified compounds were 8.42% for fatty acids and 64.23% for triterpenoids and steroids.

As shown in Table 2, triterpenoids and steroids are the major compounds in the lipophilic extract of *D. grandiflorum*. They were also described to occur in other *Chrysanthemum* species, such as *C. indicum, C. macrocarpum* as well as *C. morifolium* (*D. grandiflorum*) cultivars (Takahashi and Sato, 1979; Akihisa et al., 2005; Kumar et al., 2005).

Larvicidal activity

LC_{50} values for MeOH and CH_2Cl_2 extracts were 5.02 ppm and 5.93 ppm, respectively (Table 3).

At the concentration 1 μg/ml, temephos (Trade Name: Abate®), as positive control, killed all the tested larvae. Since the CH_2Cl_2 extract was obtained as the second product from the crude MeOH extract, it is reasonable to think that the larvicidal activity of the flowers is concentrated in the more lipophilic compounds of the

Table 3
Larvicidal efficacy of extracts from *Dendranthema grandiflorum* flowers against *Aedes aegypti*.

Samples	LC_{50} (ppm) (LCL/UCL)	LC_{95} (ppm) (LCL/UCL)	LC_{99} (ppm) (LCL/UCL)
Dichloromethane extract	5.9322 (5.1476/6.7862)	12.5650 (11.0297/14.9183)	15.3131 (13.3070/18.4474)
Methanol extract	5.0220 (3.6004/6.3780)	14.5585 (12.1309/18.8137)	18.5096 (15.2542/24.3771)
Temephos	nd	nd	1.0

LCL: lower confidence limit; UCL: upper confidence limit; (–) lethal concentration not calculated.
nd: not determined.

plant. In fact, from other studied plants, our group has identified larvicidal compounds with that nonpolar characteristic, including terpenes and alkamides (Simas et al., 2004, 2007, 2013), although more polar compounds, like saponins (Amin et al., 2011), have also been described as larvicides. Lipophilic compounds are more active larvicides based on their easier transport through insect cell wall and cytoplasmic membrane (Mann and Kaufman, 2012). From the CH_2Cl_2 extract of *D. grandiflorum*, several lipophilic compounds were found, including fatty acids, steroids and triterpenes. Palmitic acid was the major fatty acid identified. Rahuman et al. (2000) tested palmitic acid as a larvicide against *Culex quinquefasciatus*, *Aedes stephensi* and *A. aegypti*. The LC_{50} values found were 129, 79 and 57 ppm, respectively. The steroids sitosterol and stigmasterol were also identified. Sitosterol, isolated from *Abutilon indicum* (L.) Sweet, was active at LC_{50} 11.5, 3.5 and 26.7 ppm, respectively, against *A. aegypti*, *A. stephensi* and *C. quinquefasciatus* larvae (Ghosh, 2013; Rahuman et al., 2008). Triterpenes seem to be the most active of all the compounds identified. One of the triterpenes in *D. grandiflorum*, α-amyrin acetate, showed effective activity against all four larval growth stages (LC_{50} 0.01–0.02 ppm) and pupa (LC_{50} 0.005 ppm) of the mosquito *Anopheles stephensi* (Kuppusamy et al., 2009). Cholesterol is one of the most important nutrients for *A. aegypti* larval development. The protein AeSCP-2 is used by mosquitoes for the transport of cholesterol. Inactivation of it kills the larvae. Kumar et al. (2010) biocomputationally evaluated the ability of triterpenes to inactivate this protein, and α-amyrin was structurally considered to be the most effective larvicide with this particular mechanism of action. The most abundant compounds in the CH_2Cl_2 extract of the flowers were α-amyrin (22%), along with β-amyrin (13.5%) and sitosterol (13.47%).

Khan et al. (2014) compared the larvicidal activity of extracts of *C. morifolium* leaves (LC_{50} 1.5 ppm) with extracts of *Parthenium hysterophorus* L. leaves (LC_{50} 1.02 ppm) and found slightly better efficiency of the latter against *Aedes albopictus* third instar mosquito larvae. Unfortunately, they did not describe the chemical composition of the compared plants. Both plants belong to the Asteraceae family; therefore, some classes of compounds are similar. *P. hysterophorus* is a source of sesquiterpene lactones (Datta and Saxena, 2001), compounds with high insecticidal, cytotoxic, antitumor, allergenic, antifeedant and phytotoxic activity, and the plant is considered a noxious weed that threatens biodiversity (Patel, 2011). Little evidence supports the presence of sesquiterpene lactones in *C. morifolium*, the chrysanthemum of florists (Schulz et al., 1975), although alantolactone was described to occur in this species (Sertoli et al., 1985). Also no environmental damage has been reported for *C. morifolium*. Pyrethrin esters comprise another compound class with high insecticidal activity. These compounds are formed by combining two acids (chrysanthemic acid and pyrethric acid) with three alcohols (pyrethrolone, cinerolone and jasmolone). They are, however, restricted to *Chrysanthemum cinerariaefolium* Vis. (Nagar et al., 2015). Using the techniques described, both sesquiterpene lactones and pyrethrins were investigated, but not found, in *D. grandiflorum*.

In a Nuclear Magnetic Resonance (NMR)-based metabolomics study, Leiss et al. (2009) were able to compare the metabolome of thrips-resistant and thrips-susceptible chrysanthemums. They concluded that the resistance was associated with higher amounts of chlorogenic acid, a monocaffeoylquinic acid, in thrips-resistant species. Phenylpropanoids are known for their inhibitory effect on herbivores and pathogens. The possible toxicity of chlorogenic acid to insects results from its oxidation product, chloroquine (Felton et al., 1991). Tunón et al. (1994) tested an ethanol extract of *Achillea millefolium* L. for antifeedant activity against the mosquito *A. aegypti*. After fractionation of the extract, chlorogenic acid at 1.2 mg/cm^2 was one of the most active compounds as a mosquito repellent, causing 100% larval mortality. Flavonoids and fatty acids, at the same concentration, were active, but with lower percentage of mortality. The greater larvicidal activity of MeOH extract of *D. grandiflorum*, when compared CH_2Cl_2 extract, which originates from it, could, therefore, result from the presence of caffeoylquinic acids and other phenolics, apart from the triterpenoids, steroids and fatty acids, as previously discussed. Based on the results of the present study, it may be speculated that the compounds, when combined, might act as a potent phytocomplex with synergistic effect, but this needs to be confirmed in future studies.

D. grandiflorum hybrid can be a source of larvicidal compounds easily extracted with methanol. Ornamental plants are abundantly cultivated in Brazilian highlands; however, after aging, they are discarded. Yet, the results of the present study demonstrate that such discarded plants could be recycled for their methanolic extracts and used to propose innovative products, as, for example, larvicides, thus helping to develop agribusiness in Brazil.

Authors' contributions

KCVWS (PhD student) collected and identified plant samples, ran the laboratory work, analyzed data, and drafted the paper. NKS, CES and GRB carried out biological studies. WR and GV performed mass spectrometry analysis and SRCS to chromatographic analysis. FGS performed a critical reading of the manuscript. AGS conducted statistical analysis. RMK designed the study, supervised the laboratory work and offered critical comments on the manuscript. All authors have read the final manuscript and approved the submission.

Conflicts of interest

The authors declare no conflicts of interest.

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References

Akihisa, T., Franzblau, S.G., Ukiya, M., Okuda, H., Zhang, F., Yasukawa, K., Suzuki, T., Kimura, Y., 2005. Antitubercular activity of triterpenoids from Asteraceae flowers. Biol. Pharm. Bull. 28, 158–160.

Amin, E., El-Hawary, S.S., Fathy, M.M., Mohammed, R., Ali, Z., Tabanca, N., Wedge, D.E., Becnel, J.J., Khan, I.A., 2011. Triterpenoidal aaponins: bioactive secondary metabolites from *Zygophyllum coccineum*. Planta Med. 77, 488–491.

Assimoupoulou, A.N., Papageorgiou, V.P., 2005. GC–MS analysis of penta- and tetracyclic triterpenes from resins of *Pistacia* species. Part I. *Pistacia lentiscus* var. Chia. Biomed. Chromatogr. 19, 285–311.

Brackmann, A., Bellé, R.A., de Freitas, S.T., de Mello, A.M., 2005. Vase life of chrysanthemum (*Dendranthema grandiflorum*) in gibberellic acid solutions. Cienc. Rural 35, 1451–1455.

Costa, H.B., Souza, L.M., Soprani, L.C., Oliveira, B.G., Ogawa, E.M., Korres, A.M.N., Ventura, J.Á., 2014. Monitoring the physicochemical degradation of coconut water using ESI-FT-ICR MS. Food Chem. 174, 139–146.

Da Silva Junior, A.G., Perez, R., Amin, D., Ferreira, M.A.S., 2011. Design and implementation of flower export consortium in Ceará state, Brazil. In: 2011 International European Forum, Innsbruck-Igls, Austria.

Datta, S., Saxena, D.B., 2001. Pesticidal properties of parthenin (from *Parthenium hysterophorus*) and related compounds. Pest Manage. Sci. 57, 95–101.

De Sá, L.Z.C.M., Castro, O.S., Lino, F.M.A., Bernardes, M.J.C., Viegas, J.C.J., Dinis, T.C.P., Santana, M.J., Romão, W., Lião, L.M., Ghedini, P.C., Rocha, M.L., 2015. Antioxidant potential and vasodilatory activity of fermented beverages of jabuticaba berry (*Myrciaria jaboticaba*). J. Funct. Foods 8, 169–179.

Felton, G.W., Donato, K.K., Broadway, R.M., Duffey, S.S., 1991. Impact of oxidized plant phenolics on the nutritional quality of dietary protein to a noctuid herbivore, *Spodoptera exigua*. J. Insect Physiol. 38, 277–285.

Ferreira, F.P.S., Morais, S.R., Bara, M.T.F., Conceição, E.C., Paula, J.R., Carvalho, T.C., Vaz, B.G., Costa, H.B., Romao, W., Rezende, M.H., 2014. *Eugenia calycina* Cambess extracts and their fractions: their antimicrobial activity and the identification of major polar compounds using electrospray ionization FT-ICR mass spectrometry. J. Pharm. Biomed. Anal. 99, 89–96.

Ghosh, A., 2013. Efficacy of phytosterol as mosquito larvicide. Asian Pac. J. Trop. Dis. 3, 252.
Khan, G.Z., Khan, I.A., Khan, I., 2014. Exploiting the larvicidal properties of *Parthenium hysterophorus* L. for control of dengue vector, *Aedes albopictus*. Pak. J. Weed Sci. Res. 20, 431–438.
Kim, Y.S., Kim, S.H., Sung, S.Y., Kim, D.S., Kim, J.B., Jo, Y.D., Kang, S.Y., 2015. Genetic relationships among diverse spray- and standard-type chrysanthemum varieties and their derived radio-mutants determined using AFLPs. Hortic. Environ. Biotechnol. 56, 498–550.
Kumar, A., Singh, S.P., Bhakuni, R.S., 2005. Secondary metabolites of *Chrysanthemum* genus and their biological activities. Curr. Sci. 89, 1489–1501.
Kumar, R.B., Shanmugapriya, B., Thiyagesan, K., Kumar, S.R., Xavier, S.M., 2010. A search for mosquito larvicidal compounds by blocking the sterol carrying protein, AeSCP-2, through computational screening and docking strategies. Pharmacogn. Res. 2, 247–253.
Kuppusamy, C., Murugan, K., Arul, N., Yasodha, P., 2009. Larvicidal and insect growth regulator effect of α-amyrin acetate from *Catharanthus roseus* L. against the malaria vector *Anopheles stephensi* Liston (Diptera: Culicidae). Entomol. Res. 39, 78–83.
Lin, L.-Z., Harnly, J.M., 2010. Identification of the phenolics components of chrysanthemum flower (*Chrysanthemum morifolium* Ramat). Food Chem. 120, 319–326.
Leiss, K.A., Maltese, F., Choi, Y.H., Verpoorte, R., Klinkhammer, P.G.L., 2009. Identification of chlorogenic acid as a resistance factor for thrips in *Chrysanthemum*. Plant Physiol. 150, 1567–1575.
Liu, N., 2015. Insecticide resistance in mosquitoes: impact, mechanisms, and research directions. Annu. Rev. Entomol. 60, 537–559.
Mann, R.S., Kaufman, P.E., 2012. Natural product pesticides: their development, delivery and use against insect vectors. Mini-Rev. Org. Chem. 9, 185–202.
Nagar, A., Chatterjee, A., Ur Rehman, L., Ahmad, A., Tandon, S., 2015. Comparative extraction and enrichment techniques for pyrethrins from flowers of *Chrysanthemum cinerariaefolium*. Ind. Crops Prod. 76, 955–960.
Nascimento, I.R., Costa, H.B., Souza, L.M., Soprani, L.C., Merlo, B.B., Romão, W., 2015. Chemical identification of cannabinoids in street marijuana samples using electrospray ionization FT-ICR mass spectrometry. Anal. Methods 7, 1415–1424.
Patel, S., 2011. Harmful and beneficial aspects of *Parthenium hysterophorus*: an update. 3 Biotech 1, 1–9.
Polanczyk, R.A., Pratissoli, D., Paye, H.S., Pereira, V.A., Barros, F.L.S., Oliveira, R.G.S., Passos, R.R., Martins Filho, S., 2008. Indução de resistência à mosca minadora em crisântemo usando composto silicatado. Hortic. Bras. 26, 240–243.
Rahuman, A.A., Gopalakrishnan, G., Ghouse, B.S., Arumugam, S., Himalayan, B., 2000. Effect of *Feronia limonia* on mosquito larvae. Fitoterapia 71, 553–555.
Rahuman, A.A., Gopalakrishnan, G., Venkatesan, P., Geetha, K., 2008. Isolation and identification of mosquito larvicidal compound from *Abutilon indicum* (Linn.) Sweet. Parasitol. Res. 102, 981–988.
Schulz, K.H., Hausen, B.M., Wallhöfer, L., Schmidt-Löffler, P., 1975. Chrysanthemenallergie. 2. Experimentelle Untersuchungen zur Identifizierung der Allergene. Arch. Dermatol. Forsch. 251, 235–244.
Sertoli, A., Campolmi, P., Fabbri, P., Gelsomini, N., Panconesi, E., 1985. Eczema caused by contact with *Chrysanthemum morifolium* Ramat. G. Ital. Dermatol. Venereol. 120, 365–370.
Simas, N.K., Lima, E.D.C., Conceição, S.D.R., Kuster, R.M., de Oliveira Filho, A.M., Lage, C.L.S., 2004. Natural products for dengue transmission control – larvicidal activity of *Myroxylon balsamum* (red oil) and of terpenoids and phenylpropanoids. Quim. Nova 27, 46–49.
Simas, N.K., Lima, E.D.C., Kuster, R.M., Lage, C.L.S., De Oliveira Filho, A.M., 2007. Potential use of *Piper nigrum* ethanol extract against pyrethroid-resistant *Aedes aegypti* larvae. Rev. Soc. Bras. Med. Trop. 40, 405–407.
Simas, N.K., Dellamora, E.C.L., Schripsema, J., Lage, C.L.S., Filho, A.M.O., Wessjohann, L., Porzel, A., Kuster, R.M., 2013. Acetylenic 2-phenylethylamides and new isobutylamides from *Acmella oleracea* (L.) R. K. Jansen, a Brazilian spice with larvicidal activity on *Aedes aegypti*. Phytochem. Lett. 6, 67–72.
Spindola, K.C.W., 2015. Cassia australis e *Dendranthema grandiflorum*: plantas ornamentais como fonte de substâncias antivirais e inseticidas e produtos de inovação. Tese de doutorado, Programa de Pós-graduação em Química de Produtos Naturais, Universidade Federal do Rio de Janeiro, Rio de Janeiro, 174 pp.
Takahashi, M., Sato, T., 1979. Studies on the components of *Chrysanthemum morifolium* Ramat. var. *sinense* Makino forma *esculentum* Makino. II. Components of one of Brands "Mottenohoka". Yakuga. Zasshi 99, 90–91.
Tunón, H., Thorsell, W., Bohlin, L., 1994. Mosquito pepelling activity of compounds occurring in *Achillea millefolium* L. (Asteraceae). Econ. Bot. 48, 111–120.
Uehara, A., Nakata, M., Kitajima, J., Iwashina, T., 2012. Internal and external flavonoids from the leaves of Japanese *Chrysanthemum* species (Asteraceae). Biochem. System. Ecol. 41, 142–149.
World Health Organization, 1970. Insecticide Resistance and Vector Control. Technical Report Series 443. WHO, Geneva.