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Artigo

Preliminary study of the molluscicidal and larvicidal properties of some essential oils and phytochemicals from medicinal plants

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RESUMO: "Estudo preliminar das propriedades moluscicidas e larvicidas de alguns óleos essenciais e fitoconstituintes de plantas medicinais". Este estudo teve como objetivo avaliar a atividade moluscicida e larvicida de alguns óleos essenciais e fitoconstituintes de plantas medicinais. A atividade moluscicida e larvicida foi determinada empregando-se, respectivamente, o teste de letalidade contra náupilos de *Artemia salina* Leach. Artemiidae e contra larvas de *Aedes aegypti* L. Culicidae. Os óleos essenciais de *Eugenia uniflora* L. Myrtaceae, *Laurus nobilis* L. Lauraceae, *Origanum vulgare* L. Lamiaceae e os fitoconstituintes α-pineno e eugenol mostraram bioatividade citotóxica frente *A. salina* com valores de CL_{50} entre 9,59 e 253,43 µL/mL. Os óleos essenciais de *E. uniflora, M. piperita, O. vulgare* e *R. officinalis* apresentaram atividade de embriotoxicidade sobre as larvas de *A. aegypti* mostrando uma variação de inibição de viabilidade entre 40 e 100%. Estes resultados demonstram o potencial de bioatividade desses óleos essenciais e fitoconstituintes e justificam, parcialmente, o desenvolvimento de estudos com esses produtos para uso popular.

Unitermos: Óleos essenciais, fitoconstituintes, *Artemia salina, Aedes aegypti,* toxicidade.

ABSTRACT: This study aimed to evaluate the molluscicidal and larvicidal activity of some essential oils and phytochemicals from medicinal plants. Molluscicide and larvicidal activity were determined by, respectively, the lethality bioassays using *Artemia salina* Leach. Artemiidae and *Aedes aegypti* L. *Culicidae larvae*. Essential oils from *Eugenia uniflora* L. Myrtaceae, *Laurus nobilis* L. Lauraceae, *Origanum vulgare* L. Lamiaceae and the phytochemicals α-pinene and eugenol presented citotoxicity toward *Artemia salina* with CL_{50} values between 9.59 and 253.43 µL/mL. Essential oils from *E. uniflora, M. piperita, O. vulgare* and *R. officinalis* showed embryotoxicity on *Aedes aegypti* larvae with a viability inhibition between 40 and 100%. These results show the bioactivity of the assayed essential oils and phytochemicals and, partially, justify their insertion in further evaluation in order to establish a safe exploitation of their biological potentiality.

Keywords: Essential oils, phytochemicals, *Artemia salina, Aedes aegypti,* toxicity.

INTRODUCTION

The toxic potential of a plant product is known as one of the main parameters to be scientifically evaluated when regarded the use of its bioactivity in classical or alternative therapies (Ruiz et al., 2005). The larvae of *Artemia salina* Leach., Artemiidae, has been used as target organism in assays focusing the detection of bioactivity of plants and derivatives, particularly their toxicity (Alves et al., 2000). Brine shrimp lethality assay is known as an efficient, safe, fast and reproducible procedure to assess biological potentiality of new compounds (Meyer et al., 1982) and requires a small amount of the assayed substance (2-20 mg) (Svensson et al., 2005). Still, it has presented satisfactory correlation with insecticidal, anti-*Trypanosoma cruzi* (Alves et al., 2000; Montanher et al., 2002) and citotoxicity property to some solid human tumors (McLaughlin et al., 1998).

Brine shrimp lethality assay has been applied in preliminary studies of toxicity with algae, plant products, dentistry materials, to screen mould toxins and exposure to heavy metals e pesticides (Ara et al., 1999; Martinez et

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al., 1999; Pelka et al., 2000; Lima et al., 2002; Ruiz et al., 2005; Lhullier et al., 2006).

A. salina Leach. is a microcrustaceous inserted in the Arthropoda division, Crustaceae class, Branchiopoda subfamily and Anostraca order. It has an 8 to 10 mm length, with coloring (light red, pink or golden brown) ranging according to its habitat (Lopes et al., 2002). The one reproduces quickly and its dried eggs can be stored for approximately ten years keeping viability to emerge when exposed to saline water (Parra et al., 2001; Montanher et al., 2002).

The dengue presents as an endemic disease in Africa, Asia, Central and South America and, in Brazil, the disease is widespread meaning a steady worrying for the public health system (Funasa, 2002; Luna et al., 2005). The discovery of new substances with potential application to control the survival and reproduction of their vector, that is *Aedes aegypti* L. Culicidae, is primordial when regarded the role that the vector plays in the disease spreading. Still, it is well established that the control of the vector survival and reproduction is the unique viable approach to avoid the dengue high prevalence (Marcondes, 2001).

Insecticides currently used to control the *A. aegypti* proliferation need a continuous application with progressively increased dosages which possibly means risk of toxicity for humans and animals. Some studies has been carried out with the purpose of discovering alternative products, including plant derivatives, with insecticidal properties to be applied for inhibiting the survival of *A. aegypti* (Luna et al., 2005).

Regarding the lack of similar studies, this research had as purpose to verify the toxicity property, particularly the molluscicide and larvicidal property, of some essential oils and phytochemicals from medicinal plants using bioassays with *A. salina* and *A. aegypt.*

MATERIAL AND METHODS

Essential oils and phytochemicals

Essential oils from *Laurus nobilis* L. Lauraceae and *Origanum vulgare* L. Lamiaceae were obtained from Ferquimica Ind. Com. Ltda. (Vargem Grande Paulista-SP, Brazil) and their quality parameters (appearance, color, purity, odor, density 20 °C, refraction index 20 °C) were described in an accompanying technical report. This provider produces essential oils by steam distillation on industrial scale. The essential oils from *Eugenia uniflora* L. Myrtaceae, *Mentha piperita* L. Lamiaceae and *Rosmarinus officinalis* L. Lamiaceae were supplied by the Laboratory of Pharmaceutical Technology, Federal University of Paraíba, João Pessoa-PB, Brazil, and they were obtained by steam distillation according to Matos (1988). All essential oils were chosen to be included in the toxicity assays based on their biological potential according to specialized literature and use in popular medicine (Table 1).

The phytochemicals α-pinene (monoterpene) and eugenol (phenolic) were supplied by the Laboratory of Pharmacotechnique, State University of Ponta Grossa, Ponta Grossa-PR, Brazil.

Table 1. Plants from which were obtained the essential oils used in the toxicity assays and their use in the folk medicine.

Plant (popular name)	Family	Part of the plant*	Use in folk medicine	References
Laurus nobilis L. (Laurus)	Lauraceae	Leaves	Analgesic, anti-acid, anti-spasmodic, anti-rheumatic, anti-dispeptic, expectorant	Oliveira et al., 2006 Conforti et al., 2006
Origanum vulgare L. (Orégano)	Lamiaceae	Leaves	Analgesic, anti-rheumatic, anti-septic, expectorant, parasiticide, laxative.	Sellar, 2002 Souza et al., 2007
Mentha piperita L. (Mint)	Lamiaceae	Leaves	Anti-spamodic, stimulanting, sinusitis, anti-piretic, diuretic, cough, asthma, analgesic, halitose, anti-emetic, parasiticide	Matos, 1998 Vidal et al., 2007
Rosmarinus officinalis L. (Rosemary)	Lamiaceae	Leaves	Anti-rheumatic, stimulating, dispeptic, antioxidant	Sant'Ana & Mancini-Filho, 1999
Eugenia uniflora L. (brazilian cherry)	Myrtaceae	Leaves and rosebuds	Odontalgia, dispeptic, onichomycosis, endoparasitosis, respiratory infections, stimulating, repellent insecticidal, halitosis	Dip et al., 2004

*Part of the plant used in the essential oil extraction

Bioassays with *Artemia salina* L.

In this assay was assessed the molluscicidal activity of the essential oils from *E. uniflora* L., *L. nobilis* L. e *O. vulgare* L. and phytochemicals alfa-pinene, beta-pinene and eugenol. For this, cysts of *A. salina* were added to flask containing sterile saline water (pH 8.5, 29 ºC) and left under artificial lighting for 24 h in order to obtain the larvae used in the assay. After that, thirteen to fifteen larvae were exposed (in sterile assay tubes) to 5 mL of the solutions, prepared in Tween 80, DMSO and sterile saline water, with different concentrations (10-1000 µg/mL) of the essential oils and phytochemicals. The suspensions were incubated for 24 h under artificial lighting. At the end

of the incubation period, the count of the number of alive and killed larvae was carried out. *A. salina* larvae cultured in Tween 80, DMSO and sterile saline water without adding essential oil or phytochemical were used as control experiments. Each assay was performed in triplicate.

The results were expressed as LC_{50} value (concentration able to kill 50% or more of the larvae). LC_{50} was found by linear regression (square equation) using a significance level of 5% ($p < 0.05$) and reliability index of 95%. For this the Probitos Statistical Methods/Microcal Origin 6.0 software was used. When found value of LC_{50} < 1000 µg/mL the assayed product was regarded as toxic bioactive compound; on the other hand, when found value of $LC_{50} \geq 1000$ µg/mL the assayed compound was regarded as non-toxic (Meyer et al., 1982; McLaughlin et al., 1998; Parra et al., 2001; Lopes et al., 2002).

Bioassay with *A. aegypti*

In this assay was assessed the embryotoxicity activity of the essential oils from *C. aromaticus* L., *M. piperita* L., *O. vulgare* L. and *R. officinalis* L. Larvae of *A. aegypti* used in this assay were supplied by the Environmental Surveillance Center, Recife city hall, Recife-PE, Brazil. The development and maintenance of the larvae (L1, L2 and L3 biological phases), pulps and adults insects were carried out according to procedure described by Silva et al. (1998). In the assay, ten larvae in the L1 phase were cultured in solutions with different concentration of the essential oils (0.8-0.2 µg/mL) prepared in DMSO using a photophase of 12 h. The larvae development was observed in intervals of 24 h of exposure using inverted microscope (LEICA). *A. aegypti* larvae cultured in DMSO and sterile distilled water without adding essential oil were used as control experiments. Each assay was performed in triplicate and the results were expressed as percent number of non-viable larvae (viability inhibition) in comparison with the number of viable larvae found in the control assay.

RESULTS

The results of the molluscicidal assays of some essential oils and phytochemicals on *Artemia salina* Leach. Artemiidae are shown in Table 2. The essential oils from *Eugenia uniflora* L. Myrtaceae, *Laurus nobilis* L. Lauraceae and *Origanum vulgare* L. Lamiaceae, and the phytochemicals α-pinene and eugenol presented toxicity toward *A. salina* being found a LC_{50} value lower than 1000 µg/mL for all of them. Essential oils from *E. uniflora, L. nobilis* and *O. vulgare* showed LC_{50} of 253.4, 89.88 and 14.91 µg/mL, respectively. LC_{50} of 9.59 and 47.16 µg/mL were found, respectively, to α-pinene and eugenol. As can be seen, there was heterogeneity among the values of LC_{50} found for the assayed products. α-Pinene, showed the smallest LC_{50} value.

The results of the larvicidal activity of some essential oils from medicinal plants on larvae of *A. aegypti* are shown in Table 3. The essential oil from *E. uniflora* and *O. vulgare* showed a 100% inhibition of the *A. aegypti* larvae viability in all assayed concentrations. The mortality toke place in a short time (2 h, data not shown) becoming impossible the observation of the larvae development in later times The fast and high mortality rate was probably established due to the interference of the assayed essential oils on the synthesis and/or chitin reabsorption in the larvae (Silva et al., 1998).

It was observed an inhibition of the larvae development between 24 to 72 h in the assays with *Rosmarinus officinalis* L. Lamiaceae and *Mentha piperita* L. Lamiaceae essential oil. In this assays the larvae were kept in L1 phase form (data not shown). *R. officinalis* essential oil when assayed at concentration of 0.2 µg/mL presented an 80% inhibition of the larvae viability, while *M. piperita* essential oil at the same concentration provided a 40% inhibition. These oils at concentration of 0.4 and 0.8 µg/mL caused a 100% inhibition of the larvae viability. Control assays using DMSO showed development of the larvae up to L2 form in 24 h and it was noted that 60% of the assayed larvae presented in development viable form.

Table 2. Molluscicidal activity of some essential oils and phytochemicals from medicinal plants on *A. salina* L. (results expressed as average value of LC_{50}).

Natural products	LC_{50} (µg/mL)
E. uniflora EO	253.43
L. nobilis EO	89.88
O. vulgare EO	14.91
α-Pinene	9.59
Eugenol	47.16

EO: essential oil

Table 3. Larvicidal activity of some essential oils from medicinal plants on *A. aegypti* larvae. Results expressed as percent inhibition of the viable larvae.

Essential oils	Concentrations (in µg/mL)			Control *	
	0.2	0.4	0.8	DMSO	water
Eugenia uniflora	100	100	100	40	40
M. piperita	40	70	100	40	40
O. vulgare	100	100	100	40	40
R. officinalis	80	100	100	40	40

*Percent of unviable cells in comparison to the total of larvae found in the control assays

DISCUSSION

Terpenic compounds, mainly monoterpenes, are found in the most essential oils (Simões & Spitzer, 2003). α-Pinene and β-pinene (monoterpenes) are found in *Origanum vulgare* L. Lamiaceae essential oil, while eugenol (phenolic) is found in *Rosmarinus officinalis*

L. Lamiaceae essential oil (Tognolini et al., 2006). Triterpenes isolated from *Euphorbia* L. species showed no toxicity on *A. salina*, while strong toxicity was noted in diterpenes (Santos et al., 2007). Meyer et al. (1982) noted toxicological property of thymol, phenolic found in *O. vulgare* essential oil, with LC of 514 µg/mL against *A. salina.*

Extract from *Eugenia uniflora* L. Myrtaceae leaves presented no bioactivity against *Biomphalaria glabrata* and *A. salina* (Alves et al., 2000) and these findings were in agreement with those found for Montanher et al. (2002). On the other hand, *Baccharis trimera* (Less.) DC. Asteraceae showed strong toxicity (LC_{50} of 72 mg/mL) on *A. salina* and it was believed to be related with the high amount of tritepernes, particularly oleanolic acid, found in the plant (Montanher et al., 2002).

Plant species presenting high amount of flavonoids and alkaloids are reported as having interesting anti-*A. salina* property showing LC_{50} as low as of 0.13 µg/mL (Pimenta et al., 2003; Cuadra et al., 2005). Still, sesquiterpenes, triterpenes, esterols, tanins e alkaloids-containing Indian plants presented LC_{50} against *A. salina* ranging from 6.9 to 579 µg/mL (Padmaja et al., 2002).

Anti-malarial and anti-septic property was found in *Lippia multiflora* Moldenke (Verbenaceae), which also showed interesting citotoxicity toward *A. salina* (LC_{50} of 1.1 µg/mL) (Ajaiyeoba et al., 2006). Randomized clinical assays with *L. multiflora* essential oil showed anti-pediculosis activity, being reported that α-pinene and β-pinene were the major compounds found in the oil (Oladimeji et al., 2000). *Minquartia guianensis* Aubl. (Olacaceae) extracts, which already showed an LC_{50} of 25.15 µg/mL on *A. salina*, are used in equatorial Amazon as antihelminthic (Marles & Farnsworth, 1989).

Erler et al. (2006) studying the biological properties of essential oils from *M. piperita* L., *Eucalyptus globulus* L. (Mietaceae), *Pimpinella anisum* L. (Apiaceae) and *Ocimum basilicum* L. (Lamiaceae) noted repellent effect toward *Culex pipens* (filariosis etiological agent). The authors reported about the possibility of using their phytochemicals as alternative products to control vector-causing diseases.

The findings of this study are in agreement with those previously noted by Luna et al. (2005) studying the biological properties of *Ziziphus joazeiro* Mart. (Rhamnaceae) and *Caesalpinia pyramidalis* Tul. (Fabaceae) where was found a correlation between the molluscicidal activity on *A. salina* and larvicidal activity toward *A. aegypti*.

The World Health Organization reports that plant extracts showing LC_{50} < 40 ppm (0.04%; 0.4 µg/mL) has some potential to be applied as molluscicidal or larvicidal compound (WHO, 1993). Facility to be prepared, water solubility and ability to impair the laying are some attributes to be regarded when analyzed a possible molluscicidal product (Clarck et al., 1997; Ruiz et al., 2005; Lhullier et al., 2006; Santos et al., 2007).

Regarding the interesting *in vitro* toxicity of the assayed essential oils on *A. aegypti* and the importance of the dengue in the public health, our results are promising, however it is necessary that complementary researches are performed focusing the possibility of their practical and rational application to impair the survival of the dengue etiological agent.

Our data reinforce the importance of plant products as source of new bioactive compounds and show the importance of preliminary bioassays as a screening of their biologic potentiality. Still regarding the results presented here, the assayed essential oils and phytochemicals could be inserted in further pre and clinical toxicological evaluation in order to establish a safe exploitation of their biological potentiality.

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