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Full Length Research Paper

Evaluation of the lethality of *Porophyllum ruderale* essential oil against *Biomphalaria glabrata*, *Aedes aegypti* and *Artemia salina*

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The essential oil of flowers and leaves of *Porophylum ruderale* (Asteraceae) was investigated for its molluscicidal and larvicidal activities, and its toxicities to brine shrimps. The essential oil was analyzed by gas chromatography-mass spectrometry (GC/MS). GC/MS analysis showed a total volatile content of 99.98% in the *P. ruderale* oil (PREO). The major components in PREO were identified as (E)-β-ocimeno (93.95%), mircene (3.37%), (Z)-β-ocimeno (1.38%) and β-pineno (0.27%). The methodology for the molluscicidal assays involved basically the immersion of *Biomphalaria glabrata* in the dechlorinated water containing 0.1% (v/v), dimethylsulphoxide (DMSO) and PREO in the concentrations of 20 to 1000 ppm. The values of LC_{10}, LC_{50} and LC_{90} were respectively 738.96, 774.82 and 812.43 for *B. glabrata*. The brine shrimp toxicity tests were conducted using second instar larvae. Stock solutions of samples were prepared by dispersion of (PREO) [1 to 1000 ppm] in 10 ml of sea water containing 1% (v/v) DMSO. The values of LC_{10}, LC_{50} and LC_{90} were respectively 571.99, 632.69 and 699.83 for *Artemia salina* (values in ppm, 95% confidence interval). LC_{50} values estimated for *Aedes aegypti* were 132.48 and 173.65 for Porto Dantas and Rockfeller, respectively.

Key words: *Phorophyllum ruderale* (Asteraceae), chemical composition, molluscicide, larvicide.

INTRODUCTION

Two of the most important health problems facing most of the tropical Latin America, including large parts of northern Brazil, are the diseases of schistosomiasis and dengue fever (Luna et al., 2005). Since a large proportion of the population living in these areas suffers from varying degrees of poverty, the discovery of plant-derived compounds that could help with the control or eradication of these diseases would be of great value, particularly if the plants concerned were readily available to those who need them most (Santos and Sant'Ana, 1999).

Dengue is a viral disease caused by a flavivirus transmitted by the mosquito, *Aedes aegypti*. Its symptoms vary from mild fever, to life threatening dengue hemorrhagic fever and dengue shock syndrome (Balankur et al., 1966). The propagation of dengue is currently a public health threat, particularly in tropical and subtropical countries (Chiu et al., 2007). Since there are no effective treatments for this disease, the most effective way to control the virus outbreak is to avoid vector spreading. Organophosphates, such as, temephos, have been used as larvicide in several countries since the 1960's. However, resistance to pesticides has guided research to find new methods intended to control *A. aegypti* breeding (Braga et al., 2004). Additionally, the synthetic insecticides are toxic and adversely affect the environment by contaminating soil, water and air. Human schistosomiasis is estimated to affect 200 million people

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around the world, causing high levels of morbidity and mortality in 74 countries in tropical and subtropical areas. Aquatic snails (the intermediate hosts in which asexual reproduction of the parasites occurs) play a major role in the transmission of the schistosomes that cause the disease. Snail control strategies are considered a priority in the reduction of such transmission (Lardans and Dissous, 1998; Tavares et al., 2007).

The search for molluscicides is of great interest for potential focal control of parasitary diseases in endemic countries, especially schistosomiasis, a disease caused by *Schistosoma mansoni.* In this specific case, the aim is to control the aquatic snail *Biomphalaria glabrata*, the major intermediate host for transmission of *S. mansoni*. Essential oils of plants may be an alternative source of larval control agents, since they constitutes a rich source of bioactive compounds that are biodegradable into nontoxic products and potentially suitable for use in integrated management programs. In fact, many researchers have reported on the effectiveness of plant essential oils against *A. aegypti* and *S. mansoni* (Chantraine et al., 1998; Cheng et al., 2003, 2004; Jantan et al., 2005; Thomas and Rao, 2004). Due to increased environmental demands, the promotion of pest control agents of botanical origin became tangible in recent years.

The genus *Porophyllum* "Asteraceae", which occurs in an area extending from the southwest of the United States to South America, have been found to contain bioactive products that display a repellent activity against adults of the red-legged grasshopper *Melanoplus femurrubrum* (Guillet et al., 1998). However, there have not been any thorough investigations on larvicidal and molluscicide effects of essential oil of *P. ruderale* (PREO). Therefore, in this study, we analyzed the constituents of PREO and studied their mosquito larvicidal effectiveness against *A. aegypti* and molluscicide effect against *B. glabrata*. In order to determine the toxicity of the latex towards non-target aquatic species, the brine shrimp (*Artemia salina*) was employed as a model assay system since it provides a convenient in-house prescreening for general cytotoxicity (Anderson et al., 1991).

MATERIALS AND METHODS

Porophyllum ruderale (Asteraceae) flowers and leaves were collected in Brazil, State of Sergipe, around the town of Areia Branca, in March 2008. *P. ruderale* botanical material was deposited at the Herbarium of Universidade Federal **de** Sergipe (Voucher ASE12115).

Essential oil extraction

The essential oil extraction was performed in Laboratório de Ensaios Farmacêuticos e Toxicidade- UFS. The essential oil was obtained by hydrodistillation in a clavenger type apparatus for 4 h. The essential oils obtained were separated from the aqueous phase and kept in freezer until further analysis.

Analytical conditions

The essential oils obtained by water distillation were analyzed by GC/MS using a Shimadzu QP5050A equipped with a DB-5MS fused silica column (30 m - 0.25 mm; film thickness 0.25 μm), under the following conditions: helium as carrier gas at 1.0 mL/min; injector split at 250°C (split ratio 1/20); detector at 280°C, column temperature program 80°C during 1.5 min, with 4°C increase per min to 180°C, then 10°C.min^{-1} to 300°C, ending with a 10 min isothermal at 300°C. The mass spectra were taken at 70 eV with scanning speed of 0.85 scan.s^{-1} from 40 to 550 Da. Percentage composition was calculated using peak normalization method. Peak identification was assigned on the basis of comparison of their retention indices relative to n-alkane homologous series obtained by co-injecting the oil sample with a linear hydrocarbon mixture. Identification of individual components of the essential oil was performed by computerized matching of the acquired mass spectra with those stored in NIST21 and NIST107 mass spectral library of the GC/MS data system, as well as by visual inspection of published spectral data (Adams, 2007).

Molluscicidal assay of the oil

A population of adult *B. glabrata* snails was maintained according to established procedures (Santos and Sant'Ana, 1999) and provided by São Cristovão-Sergipe Brazil for subsequent experiments.

The bioassay was carried out as described by Silva et al. (2005a, b) by dissolving the sample first in dimethyl sulfoxide (DMSO) and then adding dechlorinated water to give a solution of 0.1% in DMSO. Ten adult snails (9 to 16 mm in diameter) were placed in a beaker, containing 50 ml of the oil solution at appropriate concentrations (1 to 1000 ppm). Each test concentration was set in triplicate. Snails were exposed to the potential molluscicide for 24 h at room temperature and were kept under normal diurnal lighting. After 24 h, the suspension was decanted; the snails were washed with water and offered lettuce leaves as food. The tested snails were then left in water for another 72 h and at the end of this period; they were examined to assess mortality. Snails were considered dead if they either remained motion less or did not respond to the presence of food or if the shell looked discolored. In order to verify the snail susceptibility, two control sets were used: one with niclosamide and the other containing 0.1% DMSO dechlorinated water. The concentrations that kill 90% (LC_{90}), 50% (LC_{50}) and l0% (LC_{10}) of the exposed snails (that would have survived in the negative-control cultures) were estimated by probit analysis, using the Organon software package.

Brine shrimp lethality test

The brine shrimp lethality test (BST) was performed using the method of Meyer et al. (1982) and McLaughlin et al. (1991). The brine shrimp eggs were hatched in artificial sea salt water (3.8 g sea salt per litre of water) and, after an average of 2 days from hatching, the shrimp larvae were used for experimental bioassay. The brine shrimp (*A. salina* leach) toxicity tests were conducted using second instar larvae according to the method of Stock solutions of samples which were prepared by dissolving of the test material in 10 ml of seawater containing 1% (v/v) DMSO. The PREO were tested at 1 to 1000 ppm. Each cup was left at room temperature. The control was prepared with 10 ml of degassed distilled water and 50 µl of DMSO solution, to which shrimp larvae were added. Each treatment was done with three replicates.

Table 1. Essential oil composition of the flowers and leaves of *P. ruderale* characterized by GC/MS and GC-FID.

Peak	rt (min)	Compound	(%) GC-MS	(%) GC-FID	KI
1	8.3928	β-pinene	0.27	0.33	976
2	78310	Mircene	3.37	2.82	988
3	44210	(Z)-β-ocimene	1.38	1.24	1035
4	85812	(E)-β-ocimene	93.95	94.78	1047
5	483	No identifed	1.04	0.81	1092
Total	-	-	-	99.98	-

rt= Retention time; KI = retention indices in the chromatographic column.

Survivors were counted after 24 h, and the percentages of deaths at each concentration were recorded. These data were then processed using Organon software to estimate LC_{90}, LC_{50} and LC_{10} (lethal concentration that killed 90, 50 and 10%, respectively) values with 95% confidence intervals.

Larvicidal activity

Third and fourth instar larvae of *A. aegypti* (form: Porto Dantas and Rockfeller) were used in the experiment. Tested extract of *P. ruderale* essential oil (20 mg) were dissolved 1 ml of water to make a standard solution (20 000 ppm). The standard solution was used to make five 47 ml solutions ranging from essential oils and tested at 2000, 1500, 1000, 750, 500, 400, 300, 200, 100 and 50 ppm (PREO). Twenty larvae were added to the solution. A mortality count was conducted every hour for 12 h and a final count was performed 24 h after treatment. A control solution using DMSO and water (20 ml) did not show larvicidal activity. Three replicates, with 20 larvae in each, were taken for each solution and the control. Positive control with the organophosphorate Temephos (O,O'-(thio-di-4,1-phenylene)Bis(O,O-dimethylphosphorothioate), a commonly used insecticide for larvae control, was used under the same conditions as used by health programs in Brazil (1 ppm).

Statistical analysis

Probit analysis was conducted on mortality data collected after 24 h exposure to different concentrations of testing solutions used to determine the lethal concentration for 10, 50 and 90% mortality ($LC_{10,}$ LC_{50} and LC_{90}) values. 95% confidence intervals [IC_{95}] are also reported (Finney and Stevens, 1948).

RESULTS AND DISCUSSION

Five compounds, representing 99.98% of the essential oils have been identified; their retention indices and percentage composition, listed in order of elution in the DB-5MS column, are given in Table 1. The major components of PREO were identified as (E)-β-ocimene (93.95%), mircene (3.37%), (Z)-β-ocimene (1.38%) and β-pinene (0.27%). Several oils obtained from *P. ruderale* have been previously investigated, mainly liquid samples isolated directly from glandular secretory cavities. In all cases, the major components reported were monoterpenes (Guillet et al., 1998).

The biological assays were carried out using concentrations of extracts selected according to the susceptibility of the test organism employed in the assay. The PREO exhibited low molluscicidal activity with LC_{10} = 738.96, LC_{50} = 774.82 and LC_{90} = 812.43 ppm. WHO (1993) recommends that plant extracts showing LC_{50} values <40 ppm may be employed directly against mollusc populations, whilst less active extracts may very well provide sources of new lead compounds with molluscicidal activities (WHO, 1993).

On the other hand, PREO showed an LC_{90} of 240.87 ppm against *A. aegypti*, an activity that is much higher than those reported for essential oils. PREO exhibited LC_{10}, LC_{50}, LC_{90} of 60.9, 132.48, 288.134 ppm (Porto Dantas) and 72.28, 173.65, 240.87 ppm (Rockfeller), respectively.

Activity against both forms (Porto Dantas, Rockfeller) is considered one of the most important aspects for any efficient larvicide that is to be used in the control of *A. aegypti* (Tsuda et al., 1995).

In searching for new forms to control *A. aegypti* breeding, the essential oil of *Hyptis fruticosa*, *Hyptis pectinata*, and *Lippia gracilis* were tested and exhibited larvicidal effect as compared to other plants essential oils (Chantraine et al., 1998).

At higher essential oil concentrations, the larvae showed restless movement for some time and then settled at the bottom of the beakers with abnormal wagging and died slowly. The rate of mortality was directly proportional to the concentration.

Silva et al. (2008) found that the essential oils of leaves of *Herniaria fruticosa* (Lamiaceae) Salzm., *H. pectinata* (Lamiaceae) Poit., and *L. gracilis* (Verbenaceae) were toxic against *A. aegypti* larvae. Both, *H. fruticosa* and *H. pectinata* exhibited LC_{50} of 502 ± 2.70 ppm and 366 ± 2.56 ppm, respectively. Among all the three plant species tested, *L. gracilis* was the most potent larvicidal (LC_{50} 98 ± 1.99 ppm).

The values of LC_{10}, LC_{50} and LC_{90} were respectively; 571.99, 632.69 and 699.83 ppm for *A. salina*. In 2002, Padmaja and collaborators related that general toxicity activity is considered weak when the LC_{50} was between 500 and 1000 ppm, moderate when the LC_{50} was between 100 and 500 ppm, and strong when the LC_{50} ranged from 0 to 100 ppm (Padmaja et al., 2002).

Conclusion

Initial experiments indicated that essential oil of *P. ruderale*, assayed at concentrations of 1000 ppm had low activity against adult *B. glabrata* snails. The larvae of *A. aegypti* are susceptible to the composition of the essential oil evaluated herein, particularly to the essential oil of P. *ruderale*. The use of natural products may be considered as an important alternative insecticide for the control of *A. aegypti* larvae, since they constitute a rich source of bioactive compounds that are biodegradable, nontoxic and potentially suitable for use in integrated larvae management programs. However, the cost of the essential oil may also be an important factor for its implementation, which depends on the availability of the plant and its yield/ha. Such studies are currently being conducted by our research group. In accordance with the preliminary conclusions, *P. ruderale* essential oil may be used as an ecologically safe alternative larvicide. In the present study, the brine shrimp toxicity assay was used to assess toxicity to non-target aquatic species. The essential oil of *P. ruderale*, which contains mainly (E)-β-ocimene was not highly toxic to brine shrimps.

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