Received 27 December 2008; Accepted 19 February 2009

Revista Brasileira de Farmacognosia
Brazilian Journal of Pharmacognosy
19(1B): 207-211, Jan./Mar. 2009

Artigo

# Evaluation of the molluscicidal and *Schistosoma mansoni* cercariae activity of *Croton floribundus* extracts and kaurenoic acid

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**RESUMO: "Avaliação das atividades moluscicida e cercaricida (*Schistosoma mansoni*) dos extratos de *Croton floribundus* e do ácido caurenóico"**. Extratos de *Croton fluribundus* (Euphorbiaceae), ácido caurenóico e dois derivados do ácido caurenóico foram avaliados como moluscicida, cercaricida e também foi verificada a letalidade destas amostras frente a larvas de *Artemia salina* Leach. Nestes ensaios foram observadas significantes atividades moluscicida e cercaricida associadas a uma reduzida toxicidade frente ao camarão de água salgada.

**Unitermos:** *Biomphalaria glabrata*, *Croton floribundus*, *Artemia salina,* ácido caurenóico, *Schistosoma mansoni,* cercárias.

**ABSTRACT:** Lethality of the extracts of *Croton floribundus* (Euphorbiaceae), a medicinal plant from south Brazil, and of the kaurenoic acid, an isolated compound, and two of its derivatives against adult *Biomphalaria glabrata* snails, *Schistosoma mansoni* cercariae and *Artemia salina* Leach. brine shrimp larvae are reported. Both extracts and the isolated compound showed significant molluscicidal and cercaricidal activities and reduced toxicity in brine shrimp assays.

**Keywords:** *Biomphalaria glabrata*, *Croton floribundus*, *Artemia salina,* kaurenoic acid, *Schistosoma mansoni*, cercariae.

## INTRODUCTION

Schistosomiasis is a parasitic disease and endemic disease. It affects millions of people in Africa and South America (Bilia et al., 2000). In Brazil, it affects over 8 million people and about 30 million live in hazard areas due to the presence of infected snails. Schistosomiasis may damage visceral organs, especially the liver, posing risk to life. The parasitary species in Brazil, *Schistosoma mansoni*, causes the disease popularly known as "xistose" or "barriga d'água". There are different kinds of schistosomiasis, but in all cases, the reproductive cycle involves aquatic snails. The parasite multiplies into hundreds of cercariae that can penetrate the intact human skin of those who are exposed to infected waters after leaving the snails (Marston & Hostettman, 1993). Chemotherapy is a general strategy for schistosomiasis control; but another more interesting is one that interrupts the disease vital cycle by snail's or cercariae's elimination.

The use of plants with molluscicidal properties is simple, inexpensive, and appropriate for the local control of the snail vector (Marston & Hostettman, 1993). Since the discovery of highly potent saponins in *Phytolacca dodecandra* (Phytolaccaceae) berries (Marston & Hostettman, 1993), naturally occurring molluscicides have received considerable attention and the number of reports on the use of plant-derived molluscicides has increased considerably (Marston & Hostettman, 1993; Oliveira et al., 2006; Sousa et al., 2008).

The phytochemical investigation of *Croton floribundus* (Euphorbiaceae), a tree commonly known as "capixingui" or "tapixingui", led to the isolation of kaurenoic acid (2% in dried barks). *Croton floribundus* is a medicinal plant used as an anti-inflammatory (Correa, 1984). Kaurenoic acid **(1)** is known to exhibit biological activities, including antimicrobial, cytotoxic, anti-inflammatory, and antiprotozoal activities (Ghisalberti, 1997). In the present work, we describe the molluscicidal activity against adult *Biomphalaria glabrata* snails, cercaricidal activity and the general toxicity of the methanol bark extract, the ethanol and hexane leaf

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extracts of *Croton floribundus* and some isolated diterpene, kaurenoic acid (**1**) to brine shrimp.

## MATERIAL AND METHODS

### Plant material

*Croton floribundus* was collected in Maringá, PR in August 2001 and authenticated by the Herbarium, HUM, Department of Botany, Universidade Estadual de Maringá, Paraná, Brazil (sheet no. 8 406).

### Preparation of extracts

Dried leaves (1200 g) were ground into a coarse powder and macerated in hexane (**A**) and in an ethanol sequence (**B**). Both extracts were dried under reduced pressure and freeze-dried (yields of 90 g of **A** and 175 g of **B**).

Dried barks of *Croton floribundus* (2800 g) were ground into a powder and macerated in methanol. The extract was dried under reduced pressure and freeze-dried (yield of 400 g of **C**).

### Isolation of kaurenoic acid (1)

A portion of 100 g (**C**) was submitted to silica gel column chromatography (FLUKA) with hexane, hexane-dichloromethane, and dichloromethane-ethyl acetate elution. Five fractions, A-E, were isolated. Fraction B gave pure **1** (2 g) on crystallization from methanol.

### Bioassays

The extracts and the pure compounds were either dissolved or suspended in 0.2 mL dimethyl sulfoxide (DMSO)/100 mL water for molluscicidal assays and 0.5% DMSO for brine shrimp and cercaricidal assays.

The methodology for molluscicidal assays was previously reported (Bilia et al., 2000 and Hostettmann et al., 1982). The experiment involves the immersion of *Biomphalaria glabrata* snails in an aqueous solution (50 mL per snail) containing either the extracts or the compounds in appropriate concentrations for 24 h. The snails were washed and transferred to recipients with distilled water and observed for 24 h. Heartbeat was checked by microscope. Controls were prepared only with DMSO and distilled water (0.2 mL/100 mL).

Snails infected with cercariae were exposed to artificial light for 3 h and the cercariae that released in this period are concentrated by their phototropism on the top of a recipient with destilled water made black on the base. The cercariae that were collected on graduated wells (0.3 mL) had the same emergence age. Approximately 60-80 freshly emitted cercariae were placed in each well and four wells for each concentration were tested. The organisms were observed on a stereoscopic microscope in after 15, 30 and 60 minutes. The isolated diterpene, kaurenoic acid, was tested at 400, 100 and 10 ppm and controls group were prepared only with DMSO. Results are expressed in percentage in terms of destruction of the cercariae.

Brine shrimp eggs (*Artemia salina* Leach) were placed in seawater for 48 h before use. The eggs were placed in a two-compartment tank. One was covered to keep the eggs in the dark while the other was illuminated to attract shrimps through perforations on the boundary plate. After 24 h, the phototropic shrimps, which went to the illuminated compartment, were collected by pipette and incubated under illumination for 24 h at room temperature (Harborne and Dey, 1991 and Lima et al., 2002; Silva et al., 2007; Nunes et al., 2008). Shrimps were added in groups of 10 organisms in four vials with final seawater volume of 5 mL per tested concentration. Preliminary bioassay was carried out with 1000, 100, and 10 ppm after testing intermediate dosages. In order to verify the *A. salina* susceptibility, controls used only seawater or 0.5% DMSO/seawater.

The colleted data was computerized to give $LC_{10}$, $LC_{50}$, and $LC_{90}$ values determined by probit analysis.

## RESULTS AND DISCUSSION

The $LC_{10}$, $LC_{50}$, and $LC_{90}$ and corresponding

**Table 1.** Molluscicidal activity ($LC_{10}$, $LC_{50}$, and $LC_{90}$) of hexane (**A**) and ethanol (**B**) leaf extracts and methanol bark extract (**C**) of *Croton floribundus* and kaurenoic acid (**1**), on *Biomphalaria glabrata* snails, after 24 h of observation.

| Molluscicidal activity[a] | Concentration of tested material (μg/mL) | | | |
|---|---|---|---|---|
| | **A** | **B** | **C** | **1*** |
| $LC_{10}$ | 7.6 | 1.8 | 0.43 | 0.50 [0.17-2.44] |
| $LC_{50}$ | 37.4 | 14.8 | 4.2 | 1.16 [0.96-3.22] |
| $LC_{90}$ | 85.2 | 35.2 | 11.5 | 4.28 [2.34-13.9] |

[a] Sufficient concentration needed to kill 10, 50 and 90% of the snails.
* With confidence interval. [$CI_{95}$] = 95% confidence interval.

**Table 2.** Activity of different concentration of kaurenoic acid (**1**) on *Schistosoma mansoni* cercariae after 15, 30 and 60 minutes. Data are show as percentage (%) of dead cercariae.

| Duration of exposure | Concentration of **1** | | | Control group |
|---|---|---|---|---|
| | 400 (μg/mL) | 100 (μg/mL) | 10 (μg/mL) | |
| 15 min | 100 * | 96.2 [92.8-100] | 73.3 [60.8-85.7] | 4.9 [0.2-9.6] |
| 30 min | 100 * | 98.8 [97.7-99.9] | 99.5 [98.5-100] | 6.2 [1.6-10.7] |
| 60 min | 100 * | 100 * | 100 * | 8.9 [6.1-11.6] |

**Table 3.** Toxicity ($LC_{10}$, $LC_{50}$, and $LC_{90}$) of hexane (**A**) and ethanol (**B**) leaf extracts and methanol bark extract (**C**) of *Croton floribundus* and kaurenoic acid (**1**), on *Artemia salina* Leach, after 24 h of observation.

| Toxicity[a] | Concentration of tested substances (μg/mL) | | | |
|---|---|---|---|---|
| | **A** | **B** | **C** | **1** |
| $LC_{10}$ | 102.7 [53.6-151.9] | 44.8 [24.0-65.6] | 14.3 [13.1-15.5] | 13.6 [3.4-21.7] |
| $LC_{50}$ | 481.5 [436.3-526.7] | 230.7 [173.3-302.2] | 237.7 [216.2-259.3] | 659.7 [540.3-779.0] |
| $LC_{90}$ | > 1000 | 881.6 [847.0-916.2] | > 1000 | > 1000 |

[a]Sufficient concentration needed to kill 10, 50 and 90% of the *Artemia salina* Leach.
[$CI_{95}$] = 95% confidence interval.

95% confidence interval [$CI_{95}$] of organisms exposed for 24 h to *Croton floribundus* extracts and the isolated diterpenoid, kaurenoic acid, are given in Tables 1 and 3, and the results from cercaricidal assays are showed in Table 2.

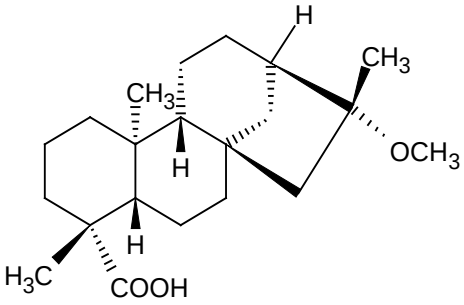
Although the molluscicidal activity is widespread in the Euphorbiaceae family, the activity varies greatly from species to species and even between different parts of the same plant (Al-Zanbagi et al., 2000). The methanol extract of barks of *C. floribundus* showed high molluscicidal activity, $LC_{50}$ at 4.2 ppm, and $LC_{90}$ at 11.5 ppm to *Biomphalaria glabrata* snails. This toxicity result is higher than others reported for other species of the Euphorbiaceae family, such as *Jatropha glauca, Euphorbia helioscopia*, and *E. schimperiana* (Al-Zanbagi et al., 2000), but neither so active as that of *Euphorbia milli* latex ($LC_{50}$ 0.12 ppm; Luna et al., 2005), which is already commonly used as molluscicides in several continents, nor that of niclosamide (BayluscideWP70®), with, $LC_{50}$ 0.077 ppm (WHO 1993). Niclosamide is known to be acutely toxic to others species (Lima et al., 2002). None of the snails in the control group died or showed significant behavioral changes.

*Artemia salina* Leach., known as brine shrimp, is a small seawater crustacean. *A. salina* larvae lethality assay is considered to be the most useful for the preliminary assessment of general activity, and the toxicity bioassays have show correlation with some cytotoxic and pesticide activities (Harborne and Dey, 1991; Shoeb et al., 2007; Subhan et al., 2008). In this work, the *A. salina* bioassays were performed to evaluate the toxicity of *Croton floribundus* extracts and kaurenoic acid against non-target organisms.

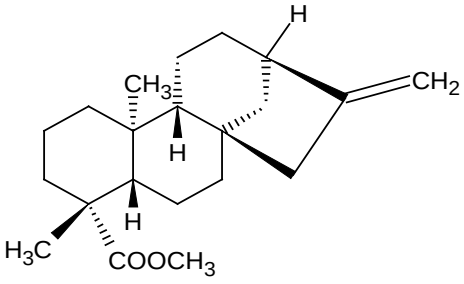
Compound **1** (Figure 1) present high activity against *Schistosoma mansoni* cercariae, killing most of the organisms, 99.5 % in 30 minutes, at 10 ppm (Table 2) and high molluscicidal activity and low toxicity for a non-target specie, *A. salina* Leach.

All *Croton floribundus* extracts and the isolated compound, kaurenoic acid, exhibited high molluscicidal activity and low lethality against non-target species, *Artemia salina* Leach. *C. floribundus* extracts present acute toxicity at $LC_{50}$ within 230-481 ppm (Table 3), while $LC_{50}$ of *Euphorbia milli* against Brine shrimp larvae was 24 ppm (Oliveira-Filho and Paumgarttem, 2000).

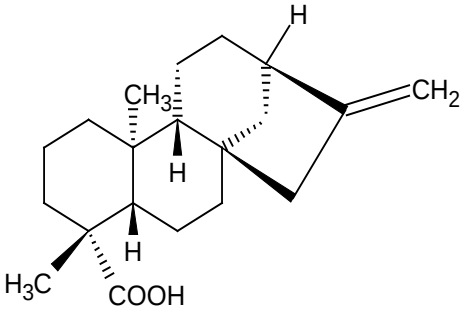
Two derivatives of kaurenoic acid were synthesized to investigate the structure/molluscicidal activity relationship, one with a methoxyl group on the double bond, 16-methoxy *ent*-kauran-19-oic acid (**2**), and another with an ester on the carboxyl group, methyl *ent*-kaur-16-en-19-oate (**3**) (Figure 1). Derivative **2** was subjected to molluscicidal evaluation at 10 and 2 ppm and showed very similar activity to that of kaurenoic acid, while derivative **3** did not exhibit any activity at the same concentrations. These experimental data suggest that the COOH group has an important relation with the molluscicidal activity of kaurenoic acid, in accordance with the observed for bidesmosidic triterpenic saponins, which have the COOH group esterified with sugar and no molluscicidal activity, while the monodesmosidics with COOH group are molluscicidal agents (Ndamba et al., 1994). Other investigations with kaurenoic acid derivatives showed that antifungal activity




**1**




**2**




**3**

**Figure 1.** Structures of compounds 1, 2 and 3.

(Boeck et al., 2005), and trypanocidal activity (Batista et al., 2007; Saúde-Guimarães & Faria, 2007) have relation with this group too.

The results observed indicate that the leaves and bark of *C. floribundus* present potent mosllucici dal agents. The activities of the leaf extracts are of interest, as they do not require killing the tree as debarking would.

## ACKNOWLEDGMENTS

The authors are grateful to Prof. Dr. Maria Conceição de Souza for the botanical identification on the plant material used. One of us (J. M.Medina) is grateful to CAPES-Brazil for doctoral fellowship.

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