Pesticide Biochemistry and Physiology xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

Pesticide Biochemistry and Physiology

journal homepage: www.elsevier.com/locate/pest

Molluscicidal activity of *Solidago canadensis* L. extracts on the snail *Pomacea canaliculata* Lam

Xiao Shen[a,c], Zhenxing Wang[a,b], Liling Liu[a,c], Zhengrong Zou[a,c,*]

[a] College of Life Science, Jiangxi Normal University, Nanchang, Jiangxi 330022, PR China
[b] College of Light Industry and Food Science, Southwest Forestry University, Kunming, Yunnan 650224, PR China
[c] Key Laboratory of Protection and Utilization of Subtropic Plant Resources of Jiangxi Province, Nanchang, Jiangxi 330022, PR China

ARTICLE INFO

Keywords:
Pomacea canaliculata Lam.
Solidago canadensis L.
Molluscicidal activity
Hepatopancreas

ABSTRACT

Extracts from the aerial parts of *Solidago canadensis* L. were evaluated for molluscicidal activity against *Pomacea canaliculata* Lam. using an immersion bioassay method. The petroleum ether fraction of the ethanolic extract (PEEE) from *S. canadensis* exhibited strong molluscicidal activity. The PEEE mode of action in the hepatopancreas tissue of *P. canaliculata* was tested at several concentrations. Biochemical parameters, namely, soluble sugar content, protein, malondialdehyde (MDA), acetylcholinesterase (AChE) activity, alanine aminotransferase (ALT), and aspartate transaminase (AST) were significantly decreased or increased after exposure to PEEE for 48 h ($p < 0.05$). Histological assessment results showed that hepatopancreas tissue structure was destroyed by exposure to PEEE. Gas chromatography–mass spectrometry analysis (GC-MS) was used to identify 15 compounds that could contribute to the molluscicidal efficacy of the PEEE. Molluscicidal assay, biochemical tests and histological assessments suggest that the PEEE from *S. canadensis* has potential utility as a molluscicide.

1. Introduction

Angiostrongylus cantonensis, the rat lungworm, is the most common infectious cause of eosinophilic meningitis in humans (Kliks and Palumbo, 1992). Most cases of *A. cantonensis* meningitis have been reported in the Asia-Pacific region (Tsai et al., 2001; McBride et al., 2017). Humans are infected by ingesting 3rd-stage larvae in raw or inadequately cooked snails, freshwater crustaceans, or fresh lettuce contaminated with the intermediate hosts (Slom et al., 2002). The larvae can migrate to the meninges and move into the spinal fluid. They move through the brain and occasionally to the eye, where they give rise to an acute inflammatory reaction rich in eosinophils. The meninges will show the main lesions, or the cerebral cortex will be involved with worm tracks, hemorrhages, and eosinophilic abscesses (Ringelmann and Heym, 1992; Monks et al., 2016).

Pomacea canaliculata Lam. (golden apple snail) is native to South America and is the major intermediate host of *A. cantonensis (Song et al., 2016)*. It was introduced into Guangzhou, China in 1981 as a food source. It has high fecundity and an adult female snail can produce > 325,000 eggs per year. It has spread widely in paddy fields and drainage ditches (Yang et al., 2010; Cowie and Barker, 2002) and has become a significant cause of eosinophilic meningitis outbreaks (Song et al., 2016). *P. canaliculata* has also caused billions of dollars of rice crop damage (Naylor, 1996), and it is a serious agricultural pest of wetland crops such as rice, taro, lettuce and semi-aquatic vegetables (Naylor, 1996; Mochida, 1991; Dai et al., 2011). Control of snail populations is important for reducing the incidence of eosinophilic meningitis in humans and reducing crop damage (Agarwal and Singh, 2010). One of the most effective methods for reducing *P. canaliculata* populations is the use of molluscicides (Agarwal and Singh, 2010; Singh et al., 1996a). Molluscicides of plant origin are often more effective, more economical, safer to non-target organisms and more readily biodegradable than synthetic molluscicides (Singh et al., 1996b; Prabhakaran et al., 2017).

S. canadensis (Asteraceae), is a medicinal plant native to North America. It was introduced into China as an ornamental plant in 1935, but it escaped cultivation and is now widely distributed and considered to be an invasive weed (Zhang et al., 2009). The chemical composition of *S. canadensis* includes phenols (Zhang et al., 2007), terpenes (Zeng et al., 2012), and essential oils (Huang et al., 2012). These plant compounds have bioactivity such as antibacterial (Mishra et al., 2010), anti-inflammatory (Tamura et al., 2009), anti-cancer (Bradette-Hebert et al., 2008), anti-tumor (Liu et al., 2007) and other effects (Arnason et al., 1981; McCune and Johns, 2002; Wangensteen et al., 2012). However, there are no reports on the effects of *S. canadensis* extracts on *P. canaliculatas* or the mode of action of these compounds in causing snail mortality.

* Corresponding author at: College of Life Science, Jiangxi Normal University, Nanchang 330022, China
E-mail address: zouzhr@163.com (Z. Zou).

https://doi.org/10.1016/j.pestbp.2018.06.009
Received 11 January 2018; Received in revised form 11 May 2018; Accepted 13 June 2018

The aim of this study was to evaluate the molluscicidal activity of extracts from the aerial parts of *S. canadensis* against the *P. canaliculata*. We also focused on the mode of action by studying the changes of selected snail enzyme activities after exposure to the extracts.

2. Materials and methods

2.1. Preparation of S. canadensis and P. canaliculatus

The aerial, non-reproductive, parts of *S. canadensis* were collected in Nanchang, China during July 2016. *S. canadensis* plants were in their growth period and the plants were about 120 cm high and 0.8 cm in diameter. Correct species identification was confirmed by Prof. Zhengrong Zou (College of Life Sciences, Jiangxi Normal University, Nanchang). A voucher specimen was deposited in the Herbarium of College of Life Science, Jiangxi Normal University, China.

Adult *P. canaliculata* (18–25 mm in length) were collected at the Shahe river in the Nanshan district, Shenzhen, China and were identified by Dr. Yankuo Li (College of Life Sciences, Jiangxi Normal University, Nanchang). A voucher specimen was deposited in the College of Life Science, Jiangxi Normal University, China. Snails were acclimatized to laboratory conditions for 72 h and fed with *Lemna minor* showing normal behavior and movement. We randomly selected snails, of the same size, for the experiments.

2.2. Preparation of S. canadensis extracts

The aerial parts of *S. canadensis* were dried outdoors under shade and ground to powder. Dried powdered plant materials (480 g) were extracted at room temperature with 70% ethanol for 12 h. The samples were then filtered. The solid residue was retrained and re-extracted an additional three times in the same manner. The filtrates were combined and then evaporated to dryness under vacuum to yield 94.76 g of ethanolic extract (19.74%, EE). A portion of this crude extract was suspended in distilled water (1000 mL) and extracted with petroleum ether, ethyl acetate and water-saturated *n*-butyl (3 × 1000 mL) to obtain a petroleum ether extract (PEEE), an ethyl acetate extract (EAEE), a water-saturated *n*-butyl extract (SBEE) and a water-residual extract (REE), respectively.

2.3. Molluscicidal assay

The molluscicidal efficacy of extracts from *S. canadensis* (EE, PEEE, EAEE, SBEE and REE) against *P. canaliculata* were tested under laboratory conditions using the snail-immersion bioassay method (Listed, 1965). We weighed a quantity of extract into dechlorinated water, then solubilized the extract with ultrasonic waves to obtain a solution or suspension of a desired concentration. The prepared solutions or suspensions were used in the molluscicidal assay. The molluscicide Mie Luo Yu Kang, sold in the agricultural market in Nanchang was used as the positive control. Mie Luo Yu Kang is a synthetic chemistry which is an off-white powder and its main chemical composition is *N*-phenyl-3-phridinecarbcexamide sulphate. The concentration of preparation of solution was 1.5 mg/mL. Ten snails were kept in a 500 mL glass beaker containing 400 mL of molluscicide at 23–27 °C. Each set of test snails were exposed to a series of concentrations of different extracts for 120 h (Table 1). The untreated control animals were kept in an equal volume of dechlorinated water under similar conditions. Each treatment was covered with a mosquito net on the top to prevent snail escape. During this experiment, the snails were not fed (feeding was not necessary during the exposure or recovery period because the snails can survive many days without apparent ill-effects (Listed, 1965)). Each treatment was replicated three times. Snail mortality was recorded at 12, 24, 48, 72, 96 and 120 h. Dead snails were removed immediately to avoid contamination of the water during the experiment. The identification of dead snails was established by stimulating the snails with a stainless steel needle and observing lack of movement (Dos Santos et al., 2003).

Table 1
Concentration of different extracts of *S. canadensis* used in toxicity trial against *P. canaliculata*.

Extracts	Quality (g)	Concentration (mg/mL)
EE	94.76	0.47, 0.94,1.88,3.76,7.52
PEEE	3.80	0.10, 0.15, 0.20, 0.25, 0.30
EAEE	8.32	1.0, 1.5, 2.0, 3.0, 3.5
SBEE	29.73	0.5, 1.0, 1.5, 2.5, 3.0
REE	33.20	1.0, 1.5, 2.5, 3.0, 3.5

$$\text{Snail mortality (\%)} = \frac{\text{number of deaths}}{\text{number of tests}} \times 100\% (= \text{number of deaths} / \text{number of snails treated} \times 100)$$

2.4. Injury to hepatopancreatic tissue

The concentrations of PEEE that killed 25% (LC_{25}), 50% (LC_{50}) and 75% (LC_{75}) of *P. canaliculata* were estimated using the Origin 8.6 software package on the basis of data from the bioassay tests. Snails were exposed to sublethal concentrations (0.11 and 0.29 mg/mL) and the LC_{50} (0.18 mg/mL) for 48 h while negative control animals were kept in an equal volume of dechlorinated water under similar conditions. Experimental methods and conditions were described in the molluscicidal assay. Each treatment was replicated four times. After 48 h of exposure, snails were randomly selected from the experimental and control glass beakers and we immediately removed hepatopancreas tissue, washed the tissue with 4 °C saline solution, dried with filter paper and weighed. Part of hepatopancreas samples from different treatments were stored in 4% paraformaldehyde for analyzing the change of microstructure. Another portion of the hepatopancreas samples was ground to a 10% (m/v) homogenate using normal saline (0.9%). The supernatant was used for the injury mechanism study after 4000 r/min centrifugation at 4 °C for ten minutes.

2.4.1. Soluble sugar content

Soluble sugar content was obtained using the anthrone-sulphuric acid colorimetry method of Van (Van, 1954), with slight modifications. Briefly, 2 mL of diluted supernatant of hepatopancreas homogenate was pipetted into a test tube. Then 4 mL of freshly prepared anthrone reagent (0.66 g of anthrone dissolved in 100 mL of 98% concentrated sulphuric acid) was added and heated in a boiling water bath for 10 min. The tubes were removed, cooled to room temperature and the absorbance of the contents was measured at 620 nm in a spectrophotometer. Amount of soluble sugar present in the sample was calculated from a standard curve drawn using variable amounts of glucose. Values were expressed as mg/g tissue.

2.4.2. Soluble protein content

Soluble protein content was estimated using the coomassie brilliant blue method (Sedmak (Sedmak and Grossberg, 1977)). One mL of diluted supernatant of hepatopancreas homogenate was added to a test tube and this was followed by addition of 5 mL of coomassie brilliant blue G-250 reagent (100 mg coomassie brilliant blue G-250 dissolved in 50 mL of 90% ethanol, added 100 mL of 85% phosphoric acid and then calibrated at 1000 mL with distilled water). After mixing by lateral shaking, the tube was undisturbed for 2 min. Absorbance was measured spectrophoto metrically at 595 nm and soluble protein was calculated from a standard curve developed using bovine serum albumin as the standard. Values were expressed as mg/g tissue.

2.4.3. Malondialdehyde (MDA) content and enzyme activity assays

The content of malondialdehyde (MDA), enzyme activity of acetylcholinesterase (AChE), alanine aminotransferase (ALT), aspartate