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ORIGINAL PAPER

Active compounds of stem bark extract from *Schima superba* and their molluscicidal effects on *Pomacea canaliculata*

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Abstract *Pomacea canaliculata* is an invasive snail that can adversely affect biodiversity, agricultural crop yields and human health. *Schima superba*, which belongs to the Theaceae family, exhibits insecticidal and antibacterial activity, but few studies have focused on its potential molluscicidal activity. In this study, we systematically evaluated the molluscicidal activity of *S. superba* extract against *P. canaliculata* in a variety of conditions, including multiple exposure intervals (24, 48 and 72 h), temperatures (15, 20 and 25 °C) and shell diameters (1.5 ± 0.2, 2.5 ± 0.2 and 3.5 ± 0.2 cm). The molluscicidal activities of a 10% microemulsion of *S. superba* extract were tested on rice in plastic pots filled with mud at different exposure intervals (1, 3, 5 and 7 days). In addition, we isolated and purified the active components from *S. superba* extract and described them. *S. superba* was highly toxic to *P. canaliculata*; the LC_{50} values for the crude extract at 72 h were 12.25 mg L^{-1} (15 °C), 11.31 mg L^{-1} (20 °C), 8.33 mg L^{-1} (25 °C) with 2.5 ± 0.2 cm shell diameter and 9.43 mg L^{-1} (1.5 ± 0.2 cm shell diameter), 9.48 mg L^{-1} (2.5 ± 0.2 cm shell diameter), 10.06 mg L^{-1} (3.5 ± 0.2 cm shell diameter) at 25 °C. There was a positive correlation between the molluscicidal activity of the extract and treatment temperature after 24 h of treatment, but there was no clear correlation beyond 24 h. In addition, there was no statistically significant correlation between the molluscicidal activity of the extract and shell diameters. Pot trials showed that the crude extract effectively controlled *P. canaliculata* at 8 and 10 mg L^{-1}. We isolated and characterized two pentacyclic triterpenoid saponins from *S. superba*, maetenoside B and sasanquasaponin III, which had high molluscicidal activity. This study provides an important foundation for future research on the potential value of *S. superba* as a molluscicide.

Keywords *Pomacea canaliculata* · *Schima superba* · Molluscicidal activity · Maetenoside B · Sasanquasaponin III

Key message

- *S. superba* has insecticidal and antibacterial activity, but it is unknown whether it has molluscicidal activity.
- The molluscicidal activity of *S. superba* extract against *P. canaliculata* was systematically evaluated at different exposure intervals, temperatures and shell diameters.
- A 10% microemulsion of *S. superba* extract effectively controlled *P. canaliculata* in pots filled with rice.
- Four compounds from *S. superba* were tested for their potential toxicity to *P. canaliculata*, and sasanquasaponin III and maetenoside B had high molluscicidal activity.

Introduction

Pomacea canaliculata is a harmful species native to the Amazon River of South America (Yan 2013). It has been an invasive species in China since the 1980s and due to its

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rapid reproduction rate, quickly spread to most of the southern provinces of China (Karraker and Dudgeon 2014). *P. canaliculata* can harm biodiversity, agricultural crop yields and human health (Zhang and Fang 2008). Therefore, it was classified as a perennial threatening species by the State Environmental Protection Administration of China in 2003 (Jiang and Yang 2006).

Chemical control is one of the most important methods to regulate *P. canaliculata*, mainly through the application of the molluscicides: niclosamide and metaldehyde (Palis et al. 1997; Attademo et al. 2016). However, these agents have detrimental effects on other aquatic organisms and irritate human skin at the concentrations required for effective molluscicide activity (Takougang et al. 2007; Hallett et al. 2015). Moreover, long-term use of a single molluscicide increases the risk of resistance (Zhong 2015). Therefore, it is urgent to explore new control strategies and their molluscicidal effects.

Screening of plant metabolites is an effective approach to identifying molluscicides. In 1933, Archibald (1933) found that extracts from the fruit of *Balanites aegyptiaca* had high molluscicidal activity. Since then, plant metabolites have been widely used against snails (Shoaib et al. 2010; Sousa et al. 2015; Yin and Jia 2016). To date, more than 1500 species of plants, mainly from the families of Theaceae, Meliaceae, Apocynaceae, Euphorbiaceae, Leguminosae, Phytolaccaceae, Solanaceae and Rutaceae, have been assessed for potential molluscicidal activity (Baptista et al. 1994). Plant species from the Theaceae family have been widely studied because of their high molluscicidal activity. Extracts from camellia, a species from the Theaceae family, have been successfully developed for use as molluscicides in China. The main active compounds of camellia are saponins (Wang et al. 2011; Xian et al. 2012).

Schima superba Gardn. & Champ is widely distributed across Southwestern China (Xu 2009). Previous investigations have shown that extracts of *S. superba* have antifeeding effects on the cabbage army worm butterfly (Deng et al. 2007, 2010, 2013). *S. superba* extracts also display some bioactivity against plant pathogens, such as *Pyricularia oryzae* Cav (Huo et al. 2008; Feng et al. 2012). However, studies have not focused on the potential of *S. superba* as a control agent against snails, except Tuo et al.'s (2005), which utilized the methanol extract of *S. superba* to control *P. canaliculata*. In this study, we systematically assessed the molluscicidal activity of *S. superba* extract against *P. canaliculata* and isolated, purified and analyzed the active ingredients from the plant extract to provide a scientific basis for the development and application of *S. superba* extracts as plant molluscicides.

Materials and methods

Isolation and structural analysis of stem bark compounds from *S. superba*

Plant material and extraction of stem bark compounds

Stem bark from *S. superba* was collected from wild populations in Yaan City, Sichuan Province, China (29.90°N, 102.92°E) in April 2015. The samples were ground using a powder grinder and dried in a drying oven at 70 °C. Bark compounds were extracted from 5.0 kg of bark powder using 25 L of extraction mixture (ethanol/distilled water, 4:1, V/V) at 75 °C for 6 h, which was repeated three times. The organic solvent was combined and concentrated at 60 °C using a rotary evaporator (Rotavapor R-205, BÜCHI, Germany). A total of 0.73 kg of crude extract was harvested.

Isolation of stem bark compounds

The 0.5 kg of crude extract was suspended in 15 L of H_2O. The crude suspension was absorbed by D101 resins (5 kg) for 12 h; then, the resins were eluted with 70% ethanol. The eluted solution was concentrated in a drying oven at 50 °C for 8 h, and the concentrated extract was separated into six fractions (Fr. YCP1-YCP6) using a C18 column (40–60 μm) and eluted with different mixtures of methanol and water (2:8, 4:6, 5:5, 6:4, 8:2 and 10:0, V:V) over time.

For the molluscicidal assay, the YCP5 fraction was further separated using a high-performance liquid chromatograph (HPLC) (Agilent, 1200 series, Waldbronn, Germany) equipped with a Phenomenex C18 column (250 × 10 mm, Phenomenex; Aschaffenburg, Germany) and a UV detector (215 nm). The mobile phases included methanol and water (70:100, V:V) to harvest the compounds YCP5-1, YCP5-2, YCP5-3 and YCP5-4. These compounds were purified by UV detection (chromatographic conditions: 30 °C; flow rate, 1 mL min^{-1}; UV detection, 210 nm; mobile phase, methanol/water ratio of 7:3, V:V).

Structure elucidation

The metabolite structures were identified using nuclear magnetic resonance (NMR) and mass spectral data (^1H and ^{13}C NMR spectra on a Bruker DRX 500 spectrometer and EI-MS on an Agilent 1290/6460 Triple Quadrupole LC/MS).

Molluscicidal assay of stem bark compounds

Animal material

Adult snails (*P. canaliculata*) in three size classes (1.5 cm ± 0.2 cm, 2.5 cm ± 0.2 cm and 3.5 cm ± 0.2 cm shell diameter, as measured using vernier caliper) were collected in May 2015 from rice fields in Wenjiang District, Chengdu City, Sichuan (30.71°N, 103.87°E). The snails were kept in plastic containers (40 cm length × 25 cm width × 30 cm height) containing 10 L of distilled water (Pall Cascada, America). The containers were placed in the laboratory and covered with a nylon net to allow ventilation. Cabbage (*Brassica oleracea*) leaves were used as a foodstuff. The snails were acclimatized to the laboratory conditions for 5 days before experiments, and dead snails were removed daily.

10% Microemulsion (ME) of extract from S. superba

The ME contained 10% stem bark extract, 10% n-butanol, 30% methyl alcohol, 40% water and 10% agricultural emulsifier 200. The mixture was stirred at 800 rpm at 40 °C until the solution turned transparent and homogeneous. The ME was developed independently by the biorational pesticide laboratory of Sichuan Agricultural University.

Assay methods

The molluscicidal activity of isolated fractions and compounds was examined using the method of Tuo et al. (2005). Bioassays were carried out using *P. canaliculata* of uniform shell size (2.5 cm ± 0.2 cm shell diameter). The tested compounds were added to 400 mL of distilled water in a 500-mL glass beaker to obtain a final concentration of 20 mg L^{-1}. Distilled water without compounds was used as a negative control. Twenty snails were placed in each 500-mL glass beaker, which was covered with a clean nylon net and incubated at 15, 20 and 25 °C in a thermostat-controlled water bath (typically, temperature-controlled water bath has a margin of error). The assay had four replicates per treatment. The mortality percentages were recorded after 24, 48 and 72 h. Dead snails were identified by the lack of response after stimulation with a stainless steel needle, at which point they were removed from the test solutions and kept in distilled water for 24 h to confirm snail mortality. The corrected mortality percentages were assessed using Abbott's formula. The data were analyzed with the software Data Processing System (DPS) 6.55. Means data were compared using Duncan's multiple range test at $P \leq 0.05$.

Determination of median lethal concentration (LC_{50})

The median lethal concentrations (LC_{50} at 24 h, 48 h and 72 h) of the extract from *P. canaliculata* were determined based on the molluscicidal activity test at different temperatures and sizes. The temperatures used were 15, 20 and 25 °C, and the snail shell sizes were 1.5 cm ± 0.2 cm, 2.5 cm ± 0.2 cm and 3.5 cm ± 0.2 in diameter. The molluscicidal activity was assessed as described above using distilled water and niclosamide as negative and positive controls, respectively. A regression equation was devised using the ratio of extract concentration and snail corrected mortality percentages to calculate the lethal concentration.

Molluscicidal activity against P. canaliculata in pots

Trials were carried out on rice in the pot-fields. Rice seedlings were transplanted into plastic pots (1 m length × 1 m width × 0.3 m height) filled with mud and covered with a nylon net to prevent snails from escaping, and 30 snails (2.5 cm ± 0.2 cm in shell diameter) were mixed into the mud in each pot. To test molluscicidal activity, 10% ME containing approximately 2, 5, 8 or 10 mg L^{-1} stem bark methanol extract of *S. superba* (the liquid level was 3 cm higher than mud level) was poured into each pot. The negative control was distilled water, and the positive control was niclosamide at concentrations of 0.5 and 2 mg L^{-1}. For each assay, there were three replicates and a randomized block arrangement. The number of living snails was counted after 1, 3, 5 and 7 days of treatment. The control efficiency was then calculated using Abbott's formula. The data were analyzed with the software Data Processing System (DPS) 6.55. Means data were compared by Duncan's multiple range test at $P \leq 0.05$.

Results

Isolation and structural analysis of stem bark compounds

YCP5-1: White powder in CH_3OH; the spectral data were similar to those in a previous report (Matsuda et al. 2010), and the structure of YCP5-1 (Fig. 1) was thus determined to be sasanquasaponin III, with a molecular formula of $C_{59}H_{94}O_{26}$.

YCP5-2: White powder in CH_3OH; the spectral data were similar to those in a previous report (Koike et al. 2001), and the structure of YCP5-2 (Fig. 2) was thus determined to be maetenoside B, with a molecular formula of $C_{59}H_{94}O_{25}$.

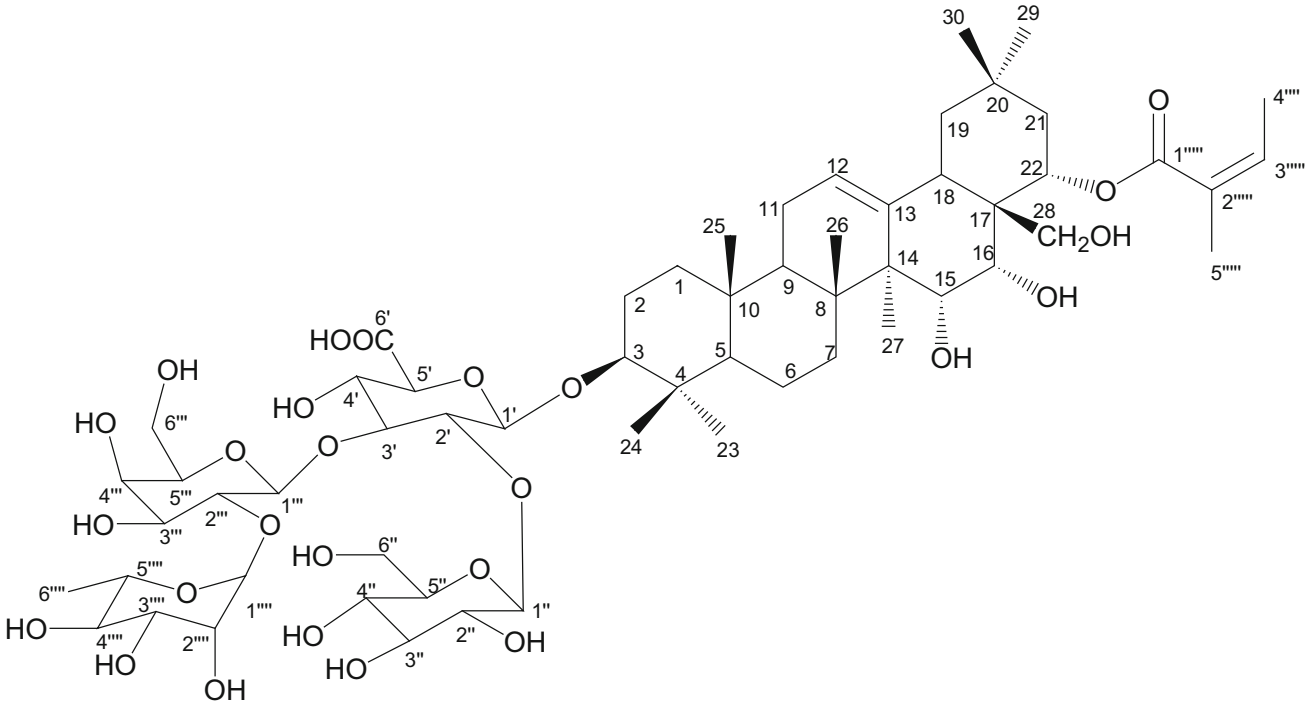

Fig. 1 Chemical structure of sasanquasaponin III

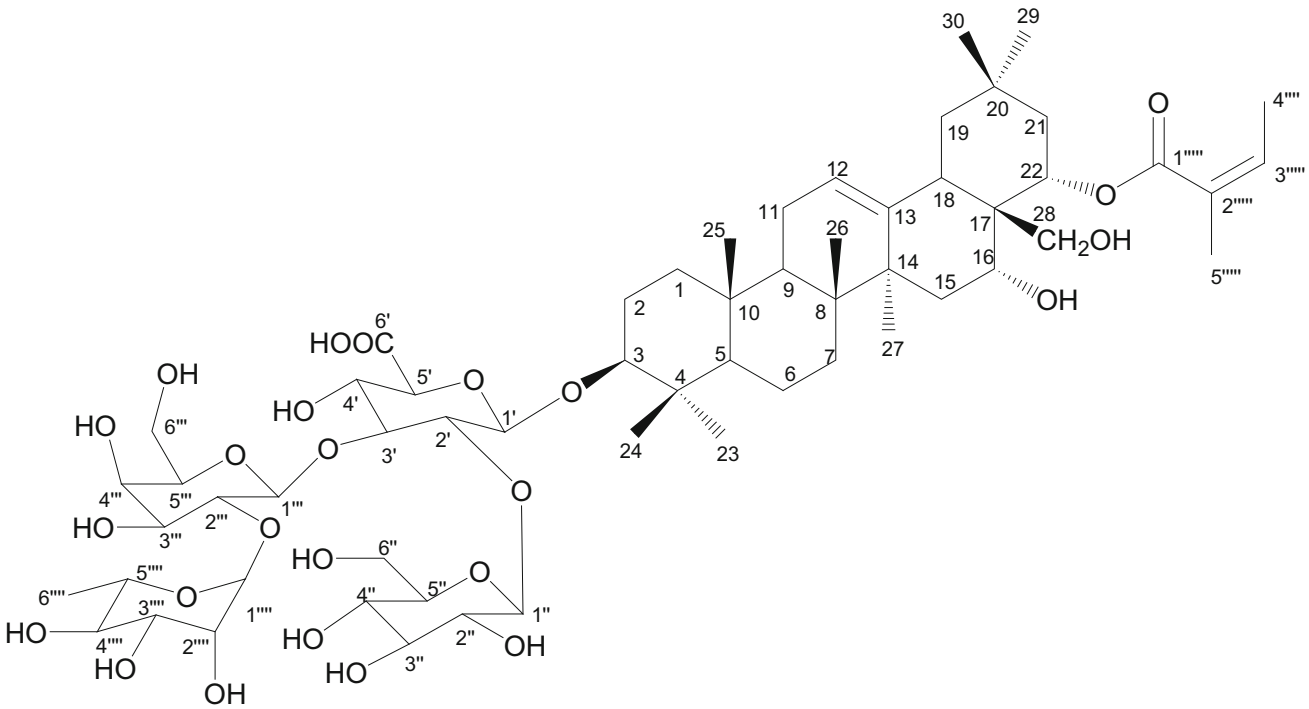

Fig. 2 Chemical structure of maetenoside B

YCP5-3: Light-brown powder in CH_3OH; the spectral data were similar to those in a previous report (Stark et al. 2005), and the structure of YCP5-3 (Fig. 3) was thus determined to be procyanidin B_2, with a molecular formula of $C_{30}H_{26}O_{12}$.

YCP5-4: Light-brown in CH_3OH; the spectral data were similar to those in a previous report (Nechepurenko et al. 2008), and the structure of YCP5-4 (Fig. 4) was thus determined to be (−)-epicatechin, with a molecular formula of $C_{15}H_{14}O_6$.

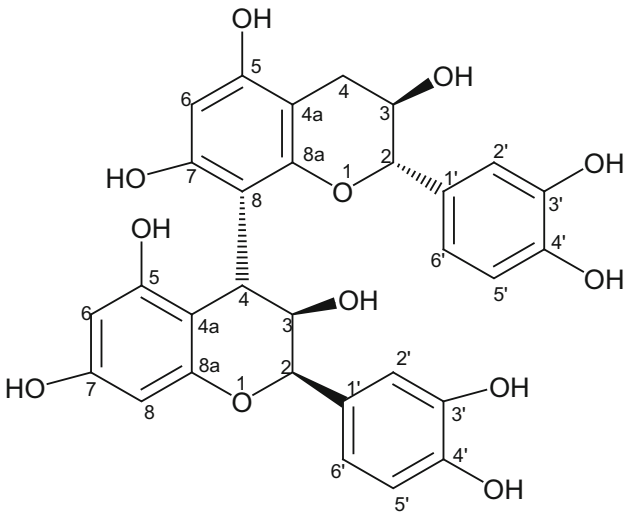

Fig. 3 Chemical structure of procyanidin B_2

Molluscicidal activity of crude extract at different temperatures

The crude extract from *S. superba* had high molluscicidal activity against *P. canaliculata* with 2.5 ± 0.2 cm shell diameter at all temperatures tested (Table 1). At 15 °C, the LC_{50} values were 31.45, 14.70 and 12.25 mg L^{-1} after 24 h, 48 h and 72 h, respectively. The control niclosamide LC_{50} values were 2.36, 1.77 and 1.59 mg L^{-1} at the same treatment times. At 25 °C, the LC_{50} values were 18.79, 8.83 and 8.33 mg L^{-1} after 24 h, 48 h and 72 h treatment, respectively. The niclosamide LC_{50} values were 1.86, 1.61 and 0.90 mg L^{-1} at the same treatment times. There was a positive correlation between the molluscicidal activity of the crude extract and treatment temperature after 24-h treatment. However, there was no clear correlation for treatments beyond 24 h.

Molluscicidal assay with different size snails

The crude extract from *S. superba* had high molluscicidal activity against *P. canaliculata* with different shell diameters (Table 2). For the smaller snails (1.5 ± 0.2 cm shell diameter), the LC_{50} values were 16.60, 9.57 and 9.43 mg L^{-1} after 24, 48 and 72 h of treatment, respectively. For the larger snails (3.5 ± 0.2 cm shell diameter), the LC_{50} values after 24, 48 and 72 h were 19.41, 11.18 and 10.06 mg L^{-1}, respectively. There was no statistically significant correlation of the molluscicidal activity of the crude extract with snail shell size.

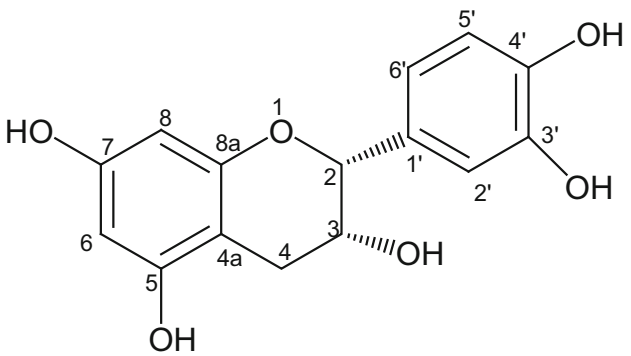

Fig. 4 Chemical structure of (−)-epicatechin

Molluscicidal activity of compounds from *S. superba*

Four compounds from *S. superba* were tested for their potential toxicity against *P. canaliculata*. Sasanquasaponin III and maetenoside B had high molluscicidal activity, with 100% mortality after 48 h of treatment with 20 mg L^{-1}. Procyanidin B2 and (−)-epicatechin had no molluscicidal activity (Table 3).

Field trial for stem bark methanol extract of *S. superba* against *P. canaliculata*

The molluscicidal activities of a 10% ME of methanol extract of *S. superba* stem bark were tested in the field (Table 4). Control efficiency against *P. canaliculata* increased significantly with the increasing concentrations of bark methanol extract of *S. superba* and increasing treatment time. Effective control of *P. canaliculata* occurred at 8 and 10 mg L^{-1}, a much higher concentration than the 0.5 mg L^{-1} used for treatment with niclosamide. No phytotoxicity to the rice plants was observed in the pot-field trials.

Discussion

Screening the toxicity of candidate plants on snails is an important way to discover novel molluscicides. Thousands of plants have been tested for their molluscicidal activity, but very few of them have potential value as molluscicides (Kloos and McCullough 1987). Among potential molluscicidal plants, *Camellia oleifera* of the Theaceae family has been developed as a molluscicide in China (Tuo et al. 2005). *S. superba*, which also belongs to the Theaceae family, is widely distributed in the Sichuan, Yunnan and Guizhou Provinces of China. In previous studies, *S. superba* exhibited insecticidal and antibacterial activity, but few reports have examined its toxic effects on snails. We found that *S. superba* exhibited high toxicity against *P. canaliculata*, with LC_{50} values of the crude extract of 9.43 mg L^{-1} (shell diameter: 1.5 ± 0.2 cm) and 10.06 mg L^{-1} (shell diameter: 3.5 ± 0.2 cm) at 72 h in the laboratory. Pot trials also showed that the crude extract effectively controlled *P. canaliculata* at 8 and 10 mg L^{-1}. These results demonstrate the potential value of *S. superba* for the control of *P. canaliculata*.

Table 1 Toxicity of niclosamide and stem bark extract from *S. superba* against *P. canaliculata* with 2.5 ± 0.2 cm shell diameter at different temperatures (15, 20, 25 °C) after 24 h, 48 h and 72 h of treatment

Temperature (°C)	Treatment (concentrations, mg L^{-1})	Treatment time (h)	LC_{50} (mg L^{-1})	95% CL (mg L^{-1})
15	Crude extract (6,9,12,15,18)	24	31.45 a	15.29–64.07
		48	14.70 c	11.73–18.42
		72	12.25 cd	10.42–14.41
	Niclosamide (0.5,1.0,1.5,2.0,2.5)	24	2.36 f	1.65–3.44
		48	1.77 f	1.42–2.20
		72	1.59 f	1.31–1.91
20	Crude extract (6,9,12,15,18)	24	19.07 b	14.42–25.23
		48	14.37 cd	12.15–16.98
		72	11.31 de	9.78–13.35
	Niclosamide (0.5,1.0,1.5,2.0,2.5)	24	1.97 f	1.44–2.69
		48	1.65 f	1.30–2.11
		72	1.03 f	0.78–1.37
25	Crude extract (6,9,12,15,18)	24	18.79 b	13.66–26.04
		48	8.83 e	7.38–10.57
		72	8.33 e	7.09–9.69
	Niclosamide (0.5,1.0,1.5,2.0,2.5)	24	1.86 f	1.54–2.25
		48	1.61 f	1.28–2.03
		72	0.90 f	0.74–1.12

Values with the same lowercase letters indicate no statistically significant differences

Table 2 Toxicity of niclosamide and stem bark extract from *S. superba* against *P. canaliculata* of different shell sizes (1.5 ± 0.2 cm, 2.5 ± 0.2 cm, 3.5 ± 0.2 cm) at 25 °C after 24, 48 and 72 h of treatment

Shell diameter of snail (mm)	Treatment	Treatment time (h)	LC_{50} (mg L^{-1})	95% CL(mg L^{-1})
1.5 ± 0.2 cm	Crude extract (6,9,12,15,18)	24	16.60 b	12.69–21.77
		48	9.57 c	8.30–11.04
		72	9.43 c	8.15–10.91
	Niclosamide (0.5,1.0,1.5,2.0,2.5)	24	0.99 d	0.75–1.33
		48	0.90 d	0.70–1.17
		72	0.87 d	0.68–1.13
2.5 ± 0.2 cm	Crude extract (6,9,12,15,18)	24	18.56 ab	13.89–24.76
		48	9.90 c	8.81–11.15
		72	9.48 c	8.38–10.74
	Niclosamide (0.5,1.0,1.5,2.0,2.5)	24	1.38 d	1.08–1.75
		48	1.30 d	1.02–1.64
		72	1.23 d	0.97–1.58
3.5 ± 0.2 cm	Crude extract (6,9,12,15,18)	24	19.41 a	13.61–27.64
		48	11.18 c	9.66–12.84
		72	10.06 c	8.85–11.45
	Niclosamide (0.5,1.0,1.5,2.0,2.5)	24	1.62 d	1.27–2.08
		48	1.39 d	1.12–1.76
		72	1.38 d	1.10–1.72

Values with the same lowercase letters indicate no statistically significant differences

Water temperature is an important factor that affects the biological metabolism of *P. canaliculata* (Fu and Wang 1999; Pan et al. 2008; Liu et al. 2011). Previous research indicates that the appropriate living temperature for *P. canaliculata* is between 15 and 30 °C. The growth rate, feeding ability and survival rate of *P. canaliculata* decrease

Table 3 Molluscicidal activity of four compounds (sasanquasaponin III, maetenoside B, procyanidin B2, (−)-epicatechin) from *S. superba* after 24, 48 and 72 h of treatment

Compound	Corrected mortality (%)		
	24 h	48 h	72 h
Sasanquasaponin III	81.25 a	100	100
Maetenoside B	70 b	100	100
Procyanidin B2	0 c	0	0
(−)-Epicatechin	0 c	0	0
Negative control	0 c	0	0

Values with the same lowercase letters indicate no statistically significant differences

at temperatures below 15 °C or exceeding 30 °C (Pan et al. 2008). In this study, we designed three temperature gradients (15, 20 and 25 °C) to avoid temperature factor-induced *P. canaliculata* death and researched the toxic effect of *S. superba* extract on *P. canaliculata* at different temperatures. We observed a significant decrease in the molluscicidal activity of the extract from *S. superba* with decreasing temperature at 24 h after treatment. However, at treatment times longer than 24 h, there was no clear difference in molluscicidal activity with temperature. Thus, the sensitivity of *P. canaliculata* to *S. superba* extract was dependent on the specific time and temperature, namely the sensitivity was enhanced with increasing temperature, but the difference gradually decreased with prolonged time. Mollusk respiration primarily occurs by exchange of dissolved oxygen in water, and within a certain temperature range, the oxygen demand increases with temperature, resulting in more frequent exchange (Li et al. 2011). More frequent water exchange may have increased the entry of the *S. superba* extract into the body of *P. canaliculata* at high temperature, thus hastening the death of the snails. However, as time increased, the *S. superba* extract accumulated in the body of the snails to a constant level, resulting in the apparent decrease in sensitivity.

The shell diameter of *P. canaliculata* is an important biological indicator for the snail's growth stage. Differences in resistance and sensitivity to external stresses have been observed in snails in different growth stages. Dai et al. (2011) found that the molluscicidal efficacy of *Nerium indicum* cardiac glycosides against *P. canaliculata* was closely related to snail size. The molluscicidal efficacy was strongest when the shell diameter was <10 mm, followed by shell diameter ≥40 mm, but lowest when the shell diameter was ≥20 mm but <30 mm. Xian et al. (2012) studied the effect of tea saponin on the growth of *P. canaliculata* at different developmental stages, which showed that the molluscicidal activity decreased as the snails aged. We found that the molluscicidal activity of the crude extract did not change with snail shell size, indicating that *P. canaliculata* was extremely sensitive to the *S. superba* extract and that the molluscicidal efficacy was not influenced by snail shell size. In this study, we only employed three size classes of *P. canaliculata*, and the molluscicidal activity of *S. superba* extract against juvenile snails or snails of other shell diameters should be evaluated in further experiments.

Previous reports have shown that saponins, especially pentacyclic triterpenoid saponins, are among the most important groups of molluscicidal agents due to their high activity. Among these compounds, different chemical structures show distinctly different molluscicidal activity. Here, we isolated and elucidated two pentacyclic triterpenoid saponins from *S. superba*, maetenoside B and sasanquasaponin III. Sasanquasaponin III was previously found in *Camellia japonica* and *Camellia oleifera.* Maetenoside B was identified from *Paramichelia baillonii,* as previously described by Koike et al. (2001). These two compounds have similar structures that are consistent with tea saponin compounds and have not been reported previously from *S. superba*. We found that maetenoside B and sasanquasaponin III had high molluscicidal activity, providing a basis for future studies of *S. superba* as a plant

Table 4 Pot trial results of niclosamide and 10% stem bark methanol extract of *S. superba* ME against *P. canaliculata with* 2.5 ± 0.2 cm shell diameter after 1, 3, 5 and 7 days of treatment

Treatment	Treatment concentration (mg L^{-1})	Control efficiency (% ± SEM)			
		1 day	3 day	5 day	7 day
10% stem bark methanol extract of *S. superba* ME	2	8.89 ± 1.11d	27.78 ± 1.11f	34.44 ± 1.11e	37.78 ± 1.92d
	5	32.22 ± 1.11c	43.33 ± 1.92d	50.00 ± 1.92c	53.33 ± 1.92c
	8	61.11 ± 2.94b	86.67 ± 1.11c	92.22 ± 1.11b	93.33 ± 0.00b
	10	64.44 ± 1.11b	94.44 ± 1.11b	95.55 ± 1.11b	95.55 ± 1.11b
Niclosamide	0.5	25.56 ± 2.94c	34.44 ± 1.11e	40.00 ± 1.92d	43.33 ± 0.00d
	2.0	95.55 ± 1.11a	100 ± 0.00a	100 ± 0.00a	100 ± 0.00a
Negative control		0e	0g	0f	0e

Values with the same lowercase letters indicate no statistically significant differences

molluscicide. Previous reports have demonstrated the activities of extracts from *S. superba* against insects (Deng et al. 2007) and phytopathogenic fungi (Zhang 2011). These findings suggest that maetenoside B and sasanquasaponin III might display activity against other pests. Future studies should focus on understanding the pharmacological activities of these two compounds.

Author contributions

CPY, MZ, GSG and HBC conceived and designed research. CPY, XXN, TXL and XFS conducted experiments. CPY and GZY analyzed data. CPY, HBC and XLC wrote the manuscript. All authors read and approved the manuscript.

Compliance with ethical standards

Conflict of interest All authors declare that they have no conflict of interest.

Ethical approval This article does not contain any studies with animals performed by any of the authors.

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