Acacetin-7-rutinoside from *Buddleja lindleyana*, a New Molluscicidal Agent against *Oncomelania hupensis*

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Buddleja lindleyana is a medicinally important member of the family Loganiaceae distributed in Eastern China. The plant has been used in different traditional medications for the treatment of various diseases. Acacetin-7-rutinoside was isolated from the *n*-butanol fraction of fresh *B. lindleyana* leaves and found to be a new molluscicidal agent against the snail *Oncomelania hupensis*. The structure of acacetin-7-rutinoside was elucidated based on spectral data, including ^{1}H NMR and ^{13}C NMR.

Key words: Acacetin-7-rutinoside, *Buddleja lindleyana*, Molluscicidal, *Oncomelania hupensis*

Introduction

Schistosomiasis, a disease caused by *Schistosoma* infestation of the host (Smith and Christie, 1989; Lockyer *et al*., 2003), remains a major public health problem that affects 200 million people in many parts of the developing world in Africa, Asia, and tropical America (Chitsulo *et al*., 2000; WHO, 2002). *Schistosoma japonicum* is the most common human schistosome and was once prevalent in twelve provinces in South China. Schistosomiasis has been eliminated in five provinces after 50 years of intensive control efforts (Zhou *et al*., 2005). Snails are the intermediate host, wherein the schistosomes transform from miracidiae into cercariae. Thus, *S. japonicum* could potentially be controlled by disrupting its life cycle through control of the snail host. Niclosamide, a chemical molluscicide, is effective in rapidly controlling snail populations, but it is expensive. Furthermore, niclosamide is lethal to non-target organisms such as amphibians and fish (Andrews *et al*., 1982). These issues have prompted scientists to develop new molluscicides, including molluscicidal agents of plant origin, which are cheaper and locally available alternatives to synthetic products used in snail control. Many countries are searching for safe and effective low-cost molluscicides that naturally occur in plants, and various plant products with molluscicidal activity have been identified, such as ginkgolic acids, ginkgols, arecoline, and papain (Adenusi and Odaibo, 2008; Ke *et al*., 2008; Yang *et al*., 2008; Preetee *et al*., 2008; Preetee and Singh, 2008).

Buddleja lindleyana is a medicinally important member of the Loganiaceae family and is distributed in Eastern China, including the provinces Jiangsu, Anhui, Jiangxi, and Hubei. This plant has been used in different traditional medications for the treatment of various diseases. Some compounds isolated from *B. lindleyana* include phenethyl alcohol glycosides, phenylpropanoid phenolic glycosides, sesquiterpenes, diterpenes, triterpenes, flavonoids, and further constituents (Miyase *et al*., 1991; Arciniegas *et al*., 1997). Moreover, this plant has anti-inflammatory, immunomodulatory, antidementia, neuroprotective, antibacterial, and antioxidant activities (Mensan, 2000; Yoshida *et al*., 1978).

Our preliminary results indicated that the *n*-butanol fraction of *B. lindleyana* (NFBL) has significant activ-

ity against *Oncomelania hupensis* and is safe for zebrafish (Han *et al*., 2010). Therefore, NFBL may be a potent and safe molluscicidal agent. The molluscicidal and ethnopharmacological significance of *B. lindleyana* prompted further studies on NFBL. We tested NFBL for molluscicidal compounds and subsequently isolated and identified them.

Material and Methods

Source of snails

Relatively uniformly sized (8 – 10 mm) snails (*Oncomelania hupensis*) were collected from the bank of the Yangtze River near Zhenjiang, Jiangsu Province, China, and acclimatized in the laboratory at room temperature in dechlorinated water [(25 ± 2) °C] for 1 d.

Isolation and identification of molluscicidal compound from NFBL

Buddleja lindleyana specimens, collected in the Huangshan mountains, Anhui Province, China, were identified by Prof. De-qun Wang, School of Pharmacy, Anhui University of Chinese Medcine, Hefei, China. oucher specimens have been deposited at the Pharmacognosy Laboratory, School of Pharmacy, Jiangsu University, Zhenjiang, China.

Plant materials were air-dried, then crushed into powder with a particle size below 40 mesh using a pulverizer. Powdered material (1000 g) was extracted with 95 % ethanol (10.0 L) at room temperature [(25 ± 2) °C] for 24 h and filtered. The residue was extracted twice more in a similar manner. The solvent was evaporated under reduced pressure in a rotary evaporator and the residue consecutively extracted with the solvents petroleum ether, diethyl ether, ethyl acetate, and *n*-butanol saturated with water. The *n*-butanol extract was reduced to dryness in a rotary evaporator, and the residue, designated NFBL, was stored in a freezer at −20 °C until required.

NFBL (55 g) was subjected to column chromatography over silica gel using a dichloromethane/methanol (30:1 → 0:1, v/v) gradient as eluent to produce 360 250-mL fractions. The fractions were pooled after thin-layer chromatography (TLC) analysis to produce 18 groups (A1 to A18). The compound (18.5 mg) obtained from A15 showed significant activity against *O. hupensis*. The structural characterization of the isolated compound was performed by comparing its spectral data (^{1}H and ^{13}C NMR) with those in the literature.

Acacetin-7-rutinoside: White powder. – ^{1}H NMR (DSMO, 400 MHz): $\delta = 3.16 – 3.47$ (2H-3), 3.62 (1H, s), 3.67 (1H, s), 4.47 (1H, s), 4.55 (1H, brs, H-1‴), 4.61 (1H, s), 4.71 (1H, s), 5.07 (1H, d, $J = 7.2$ Hz, H-1″), 5.22 (2H, d, $J = 8.8$ Hz, H-6″), 5.45 (1H, s), 6.46 (1H, s, H-6), 6.80 (1H, s, H-8), 6.96 (1H, s, H-3), 7.17 (2H, d, $J = 8.0$ Hz, H-3′,5′), 8.06 (2H, d, $J = 8.0$ Hz, H-2′,6′), 12.90 (1H, s, 5-OH). – ^{13}C NMR (DSMO, 400 MHz): δ = 63.4 (C-2), 104.3 (C-3), 182.5 (C-4), 157.5 (C-5), 101.0 (C-6), 164.4 (C-7), 95.3 (C-8), 161.6 (C-9), 105.9 (C-10), 123.1 (C-1′), 128.9 (C-2′,6′), 115.2 (C-3′,5′), 162.9 (C-4′), 100.4 (C-1″), 73.5 (C-2″), 76.1 (C-3″), 70.1 (C- 4″), 76.1 (C-5″), 66.5 (C-6″), 100.1 (C-1‴), 70.8 (C-2‴), 70.1 (C-3‴), 72.5 (C-4‴), 68.8 (C-5‴), 18.2 (C-6‴), 56.1 (4′-OCH_3).

Assay of molluscicidal activity

Molluscicidal activity against *O. hupensis* was tested via the immersion test method, as suggested by the WHO (1983). Twenty snails were placed in a glass bottle containing 100 mL of a solution of the molluscicidal compound. The snails were exposed to the molluscicide solution for 24, 48, and 72 h under normal diurnal lighting. Each test was performed in quadruplicate.

The solutions in the glass bottle were decanted and the snails were washed with dechlorinated water, then immersed in dechlorinated water and observed for 48 h. Finally, the snails were checked for mortality by mechanical prodding. Mortality rate (%) was expressed as the ratio of the number of killed snails to the total number of tested snails.

In the positive control group, snails were treated with 1 mg L^{-1} niclosamide, a potent molluscicide, while the negative control group was exposed to dechlorinated water only.

Acute toxicity of the molluscicidal compound to zebrafish

Twenty zebrafishes (3 cm long) were kept in 2 L water (control) or 100 mg L^{-1} of the test compound in a glass bottle for 48 h at (25 ± 2) °C. The mortality rate (%) was expressed as the ratio of the number of dead zebrafishes to the total number of tested zebrafishes. No animals died in the control.

Statistical analysis

Molluscicidal activity test data were statistically analysed using SPSS 13.0, and the result was ex-

pressed as mean ± SD. The LC_{50} and LC_{90} values were calculated through probit analysis.

Results

Acacetin-7-rutinoside (Fig. 1) was obtained as a white powder, which became orange and yellow in methanol and sulfuric acid/vanillin for a phenol. It was identified through comparison of the physical and spectral data with those in the literature (Jong *et al.*, 1995).

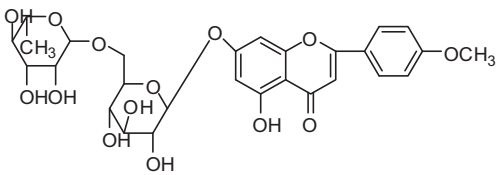

Fig. 1. Chemical structure of acacetin-7-rutinoside.

To define the toxicity of acacetin-7-rutinoside to snails, the LC_{50} and LC_{90} values at different exposure times were obtained through probit analysis of the bioassay data (Fig. 2) Acacetin-7-rutinoside showed significant activity against *O. hupensis*. The LC_{50} values were inversely correlated with exposure time and decreased from 36.12 mg L^{-1} (24 h) to 3.46 mg L^{-1} (72 h), as exposure time was increased. In the positive control group, mortality was 100%, while in the negative control group it was 0% within 24 h.

The acute toxicity of acacetin-7-rutinoside to non-target aquatic species was assessed for zebrafish. At a concentration of 100 mg L^{-1}, no zebrafish mortal-

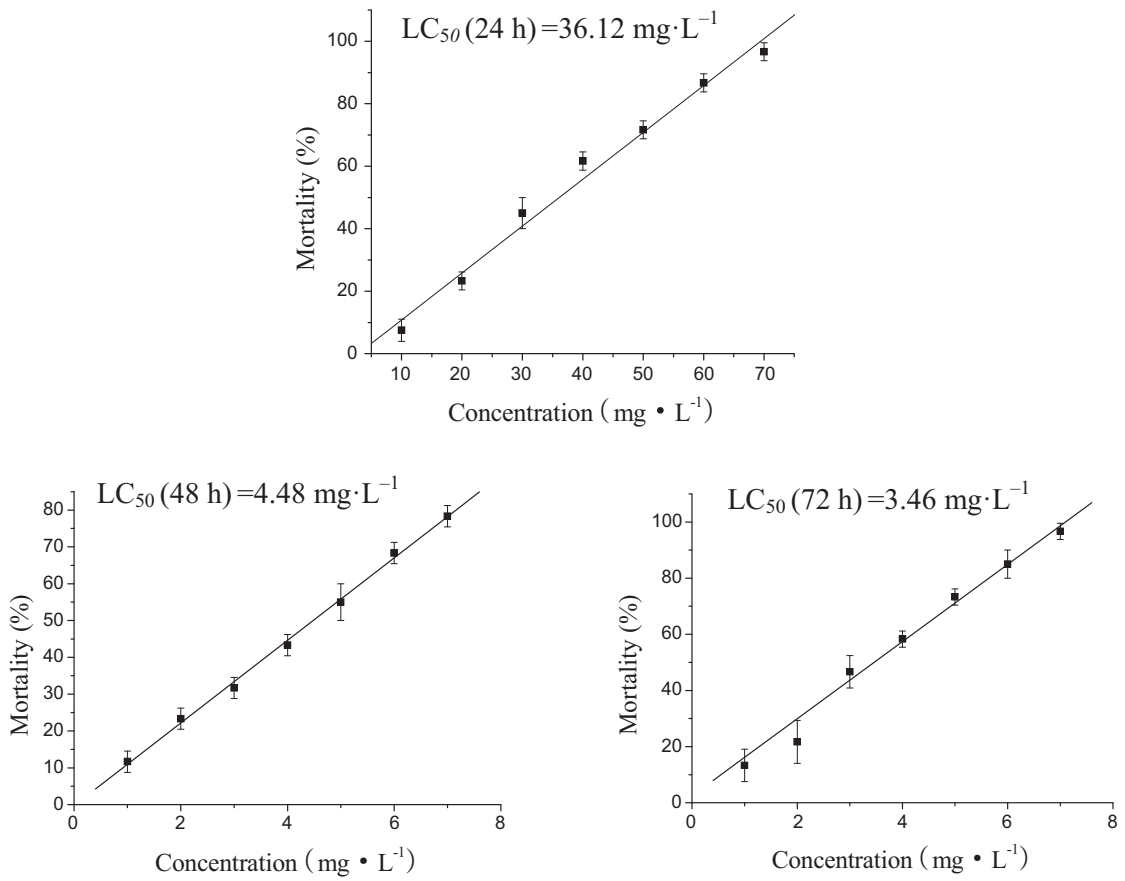

Fig. 2. Mortality of snails treated for 24, 48, and 72 h with increasing concentrations of acacetin-7-rutinoside. LC_{50} values were calculated by linear regression. (Linear regression for 24 h: $Y = -4.21736 + 1.50124 \cdot X$, $R = 0.99335$; for 48 h: $Y = -0.23571 + 11.20888 \cdot X$, $R = 0.99879$; for 72 h: $Y = 2.4106 + 13.74968 \cdot X$, $R = 0.99466$).

ity was observed in the 48-h test, demonstrating that acacetin-7-rutinoside is safe for zebrafish.

Discussion

Acacetin-7-rutinoside has significant bioactivities such as fungicidal, diuretic, anti-inflammatory (Wei *et al*., 2009), and has been isolated from *Robinia viscose* (Maksyutina, 1972), *Buddleja officinalis* (Li *et al*., 1996), *Pueraria hirsute* (Chkadua *et al*., 1997), and *Chrysanthemum indicum* (Wei *et al*., 2009). However, a molluscicidal activity of acacetin-7-rutinoside has not been reported previously. The present results clearly indicate that acacetin-7-rutinoside is the molluscicidal component of NFBL. The previously reported ingredients of *B. lindleyana* toxic to non-target aquatic species are the sesquiterpenes buddledins A – C (Yoshida *et al*., 1976). In this study, the result showed that acacetin-7-rutinoside has significant molluscicidal activity against *O. hupensis* and is safe to zebrafish. Acacetin-7-rutinoside may find application as a potent molluscicide against *O. hupensis*. Its mode of action in the snail remains to be elucidated.

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