Revista Brasileira de Farmacognosia
Brazilian Journal of Pharmacognosy
20(5): 712-717, Out./Nov. 2010
Received 19 Aug 2009; Accepted 16 Oct 2009, Available online 3 Sep 2010.

Article

# Molluscicidal activities of medicinal plants from eastern China against *Oncomelania hupensis*, the intermediate host of *Schistosoma japonicum*

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**RESUMO: "Atividades moluscicida de plantas medicinais do leste da China contra *Oncomelania hupensis*, o hospedeiro intermediário da *Schistosoma japonicum*."** Na busca por produtos naturais que podem ser utilizados para controle da esquistossomose, dezenove extratos de onze plantas medicinais do leste da China foram testados para atividade moluscicida contra o caramujo *Oncomelania hupensis*. A fração *n*-butanol das folhas frescas de *Buddleja lindleyana* Fortune, Buddlejaceae, mostrou atividade significativa contra os caracóis. A análise estatística revelou que os valores de CL50 e CL90 para a fração *n*-butanol foram 39,91 mg $L^{-1}$ e 59,28 mg $L^{-1}$ por 48 h, respectivamente. Por outro lado, a CL50 para a fração *n*-butanol para peixe-zebra foi 403,24 mg $L^{-1}$ por 48 h. Portanto, a fração *n*-butanol das folhas frescas de *B. lindleyana* poderá vir a ser um moluscicidas potente e seguro.

**Unitermos:** *Buddleia lindleyana,* molluscicidal activity, *Oncomelania hupensis.*

**ABSTRACT:** In a search for natural products that could be used to control schistosomiasis, nineteen extracts of eleven medicinal plants from eastern China have been tested for molluscicidal activity against snail *Oncomelania hupensis*. The *n*-butanol fraction of the fresh leaf from *Buddleja lindleyana* Fortune, Buddlejaceae, showed significant activity against the snails. Statistical analysis revealed that the LC50 and LC90 values for the *n*-butanol fraction were 39.91 mg $L^{-1}$ and 59.28 mg $L^{-1}$ for 48 h, respectively. Otherwise, the LC50 values for the *n*-butanol fraction to zebrafish was 403.24 mg $L^{-1}$ for 48 h. Therefore, the *n*-butanol fraction of the fresh leaf from *B. lindleyana* may be potent and safe molluscicides.

**Keywords:** *Buddleia lindleyana,* molluscicidal activity*, Oncomelania hupensis.*

## INTRODUCTION

Schistosomiasis is a disease caused by infestation of the host by species of the genus Schistosoma (Smith & Christie, 1989; Lockyer et al., 2003), remains a major public health problem affecting some 200 million people in many parts of the developing world, such as Africa, Asia, and tropical America (Chitsulo et al., 2000; WHO, 2002). *Schistosomiasis japonica*, is the most common human schistosome, was once prevalent in twelve provinces in the south of China. After 50 years of intensive control activities, schistosomiasis has been eliminated in five provinces (Zhou et al., 2005). Because snail is the intermediate host in which the transformation from miracidium to cercariae occurs, so an important strategy of schistosomiasis controling is to attack and break down the life cycle of *Schistosomiasis japonicum* through controlling of the snail. Niclosamide, a chemical molluscicides, is effective measures for the rapid control of the snail, but its use is expensive. Furthermore, side effects reported in literature include the lethal action of niclosamide on non target organisms, such as amphibian and fish (Andrews, et al., 1982). All of these problems push the scientists to focus their attention on plant molluscicides, which are cheaper and locally available alternative to synthetic products used in snail control. Many countries search for safe and low-cost molluscicides by using the naturally occurring plants that can be applied effectively in different habitats, and a large number of plant products with molluscicidal activity have been identified (Adenusi & Odaibo, 2007, 2008; Ke et al., 2008; Yang et al., 2008; Preetee et al., 2008a, b).

China possesses more than thirty thousands

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ISSN 0102-695X
DOI: 10.1590/S0102-695X2010005000018

plant species, is the third largest genetic diversity of plant species in the world, However less than twelve thousands have been evaluated with respect to their biological characteristics, and only about five hundreds have been subjected to detailed molluscicidal activities against host snail studies (Guo, 1987). Therefore plants still constitute a relatively under-utilised, and potentially very valuable, source for the further discovery of molluscicidal activities against host snail substances. The specific objective of the present study was to examine crude extracts of medicinal plants from the eastern of China for their molluscicidal activities against the snail *Oncomelania hupensis.* In addition, we will preliminarily discuss its toxicity to non target organisms.

## MATERIALS AND METHODS

### Plant materials

All plant species, shown in Table 1, collected in the Huangshan, Anhui province, China in July 2008, were identified by Prof. De-qun Wang of the Traditional Chinese medicine (TCM) Laboratory, School of Pharmacy, Anhui college of TCM . Voucher specimens are deposited in the Pharmacognosy Laboratory, School of Pharmacy, Jiangsu University.

Plant materials were air dried, then crushed into powder by a pulverizer. The particle size of powder was controlled below 40 mesh. Powdered material (100 g) was extracted with 95% ethanol (1.0 L) and at room temperature (25±2 $^{o}$C) for 24 h and filtered. The residue was extracted twice more in a similar manner. The extract was evaporated under reduced pressure in a rotary evaporator. Ethanolic extracts of tested materials were stored in a freezer at -20 $^{o}$C until required.

The ethanolic extract of *Buddleja lindleyana* Fortune, Buddlejaceae, leaf was further extracted by four different solvents one after one: petroleum ether, diethyl ether, ethyl acetate and *n*-butanol (NFBL) saturated by water, and the obtained solutions were evaporated under reduced pressure in a rotary evaporator. Extracts were stored in a freezer at -20 $^{o}$C until required. Each fraction was diluted with an appropriate volume of dechlorinated water in order to provide assay solution as required.

### Source of snails

The snails (*Oncomelania hupensis*) with relatively uniform in size (8-10 mm) were collected from the beach of Yangtze River near Zhenjiang in Jiangsu province of China, and acclimatized in the laboratory at room temperature (25±2 $^{o}$C) for 24 h.

### Molluscicidal activity assay of the extracts

Molluscicidal activity against *O. hupensis* was performed according to the immersion test method suggested by WHO (1983). For each test, twenty snails were placed in a glass bottle containing 200 mL of molluscicide solution of the extract. Snails were exposed to the molluscicide solution for 24, 48 and 72 h respectively and kept under normal diurnal lighting. Each test was set in quadruple.

At the set time, the solution in beakers was decanted and the snails were washed with dechlorinated water. The test snails were then left in dechlorinated water and observed for 48 h, and finally the snails were examined to check mortality by mechanical prodding. The ratio of killed snails to tested total snails was expressed as mortality (%). Finally, to check the snails' resistance, the validity of the test and its relevance, control group of the snails was treated with 1 ppm of Niclosamide. Negative group of the snails was treated with 100 mL dechlorinated water only.

### Screening of molluscicidal active fractions of *Buddleja lindleyana*

A 50 mg $L^{-1}$ of the five fractions of *B. lindleyana* leaf was used to treat the snails. The every twenty snails, as one group, were submerged in beakers containing the test solutions. Snails were exposed to the molluscicide solution for 24, 48 and 72 h respectively. Each test was set in quadruple.

### Concentration dependent between NFBL and climbing of the snails

Every twenty snails, as one group, were exposed to a concentration series of 70, 80, 90, 100 and 110 mg $L^{-1}$ of the NFBL for 48 h. The ratio of climbing snails to tested total snails was expressed as mortality (%). Each test was set in quadruple.

### Glycogen (Gn) and total protein (TPr) assay

Two concentrations of 103.19, 203.38 mg $L^{-1}$ of the NFBL, 40% and 80% of the LC50 value of 24 h respectively, were used to treat *O. hupensis* snails. The negative group was performed with the dechlorinated water only. Snails were randomly and equally divided into four groups. Every fifty snails, as one group, were submerged in beakers containing the test solutions. After exposure for 24 h, the snails were washed with dechlorinated water, and then left in dechlorinated water, the climbing snails were taken out from the beaker and their soft tissues were dried in oven for 24 h at 40 $^{o}$C, and then the dried soft tissues ware grounded into fine powder. Then 2 mL aqueous of 30% KOH was added to the test tube with 10 mg of the powder and incubated in boiling water for 20 min, and then cooled to room temperature. Finally 10 mL ethanol was added to the tube after cooling. Gn content was

analyzed by anthrone colorimetric method. TPr content was detected with KND-04 Kjeldahl nitrogen detection device, and calculated as: (TPr) %=6.25×N%.

**Zebrafish acute toxicity of NFBL**

Zebrafish were exposed to a concentration series of 100, 200, 300, 400, 500, 600 and 700 mg $L^{-1}$ for 48 h. Water temperature was 25±2 °C. Twenty zebrafishs were placed in a glass bottle containing 2000 mL solution of NFBL. Each test was set in quadruple. The ratio of killed zebrafish to tested total zebrafish was expressed as mortality (%). No mortalities were observed in the controls.

**Statistical analysis**

The molluscicidal activity test data were statistically analyzed by SPSS13.0, and the result was expressed as M±SD. Significantly different was analyzed by One-way ANOVA. The LC50 and LC90 values were calculated by the probit analysis.

**RESULTS AND DISCUSSIONS**

**Molluscicidal activity of the plant extracts**

The total of nineteen ethanolic extracts of various parts of eleven medicinal plants were assayed for molluscicidal activity and the lethality of 24, 48 and 72 h were shown in Table 1. In the above plants extracts, six ethanolic extracts showed medium to strong toxicities towards adult snails within 72 h exposure at concentrations of 200 mg $L^{-1}$. However, only the ethanolic extract of *B. lindleyana* leaf showed significant molluscicidal activity within 48 h exposure. It still showed medium toxicities towards adult snails within 24 h exposure. The mortality (%) values was 76.25±4.79%. Therefore, we focus our attention to the ethanolic extract of *B. lindleyana* leaf.

**Screening of molluscicidal active fractions of *B. lindleyana***

In order to screen the active fractions of *B. lindleyana,* fractions extracted by different solvents of B. lindleyana were assayed for molluscicidal activity. The mortality (%) values were shown in Figure 1. NFBL exhibited a much higher lethality than other fractions. The NFBL showed 100% mortality at concentrations of 50 mg $L^{-1}$ within 72 h.

**Molluscicidal activity of NFBL**

For detecting the toxicity of NFBL to snails further, the LC50 and LC90 values for different exposure time were analyzed through probit analysis of the bioassay data (Table 2). NFBL showed significant activity against *O. hupensis*. Noteworthy, there was a significant negative correlation between LC50 values and exposure time. When increasing the exposure time, the LC50 value of the NFBL was decreased from 257.00 mg $L^{-1}$ (24 h) to 23.08 mg $L^{-1}$ (72 h). It is the same to LC90, when increasing the exposure time, the LC90 value was decreased from 492.85 mg $L^{-1}$ (24 h) to 40.47 mg $L^{-1}$ (72 h).

Simultaneously, there was a significant negative correlation between climbing of the snails and concentration (Figure 2). When increasing the concentration, the climbing of the snails was decreased from 100% to 0%. The linear regression between climbing of the snails and concentration: Y=289-2.7 x (R=-0.98459).

**Gn and TPr content of snails' soft tissues tested**

To preliminarily study the mechanism of NFBL in snail controlling, the Gn and TPr content of snails' soft tissues were analyzed, after the submerging test of snails treated with concentrations less than the LC50 values. The results demonstrated that the Gn content decreased greatly after treatments, ranging from 23.90% to 34.25% (Figure 3). The TPr content also decreased after treatments, ranging from 7.74% to 10.45% (Figure 4). The decreasing rate was parallel to the molluscicidal activity. The stronger the molluscicidal activity, the more decrease Gn content and TPr content.

**Zebrafish acute toxicity of NFBL**

In the present study, the zebrafish toxicity assay was used to appraise toxicity to non target aquatic species. The results showed that NEBL was not highly toxic to zebrafish. The LC50 values was 403.24 mg $L^{-1}$ within 48 h (Figure 5). It is much higher than that of snail (39.91 mg $L^{-1}$). That is to say, NFBL caused 50% mortality in snails but had no effect on zebrafish at the concentration of 39.31 mg $L^{-1}$.

The present results clearly indicated that the leaf of *B. lindleyana* is an important source of botanical molluscicides, and molluscicidal active fractions is NFBL. The toxicity study revealed that the toxic effect is time as well as concentration dependant as evident from negative regression between exposure time. NFBL can decrease the Gn and TPr content in snail's soft tissues suggests that the abnormity of energy metabolism might be a factor for the molluscicidal activity (Wang, et al., 1989).

On the whole, NFBL may be used as potent molluscicides against *Oncomelania hupensis.* Nevertheless, further studies are required to determine molluscicidal active substance in the NFBL and the mechanism of action of NFBL in snail body.

**Table 1.** Plants employed in this study and the activities of ethanolic extracts against *Oncomelania hupensis* (n=4) (M±SD)

| Species | Plant parts extracted | mortality (%) with different submerged periods | | |
|---|---|---|---|---|
| | | 24 h | 48 h | 72 h |
| *Silybum marianum* | fruit | 5.00±4.08 | 10.00±3.54 | 22.5.0±6.46 |
| *Rhinacanthus nasutus* | herb | 6.25±2.5 | 25.00±3.54 | 81.25±10.31 |
| *Peucedanum praerutorum* | root | 0 | 11.25±5.45 | 51.25±8.54 |
| *Buddleia lindleyana* | leaf | 76.25±4.79 | 96.25±4.15 | 100.00 |
| | branch | 0 | 12.50±4.33 | 56.25±4.79 |
| *Acorus gramineus* | rhizome | 3.75±4.79 | 53.75±4.15 | 75.00±4.08 |
| | leaf | 2.50±2.89 | 7.50±2.50 | 56.25±6.29 |
| *Pterocarya stenopt* | leaf | 0 | 26.25±8.2 | 50.00±4.08 |
| | branch | 0 | 2.50±2.50 | 48.75±2.50 |
| *Clerodendron* | branch | 5.00±4.08 | 42.50±5.59 | 56.25±4.79 |
| *cyrtophyllum* | leaf | 0 | 57.50±5.59 | 65.00±7.07 |
| *Hemerocallis fulva* | leaf | 3.75±2.50 | 15.00±3.54 | 50.00±7.07 |
| | root | 0 | 22.5±5.59 | 76.25±8.54 |
| *Acorus gramineus* | leaf | 10.00±4.08 | 28.75±4.15 | 75.00±5.00 |
| | rhizome | 6.25±4.79 | 25.00±3.54 | 72.50±9.57 |
| *Sapium sebiferum* | fruit | 3.75±4.79 | 8.75±2.17 | 55.00±12.90 |
| *Juglis cathayensis* var. *formosana* | fruit | 0 | 30.00±3.54 | 60.00±8.17 |
| | branch | 5.00±5.77 | 0 | 52.50±8.66 |
| | leaf | 2.50±2.89 | 15.00±3.54 | 57.50±6.46 |
| Dechlorinated water | | 0 | 0 | 0 |
| Niclosamide | | 97.50±5.00 | 100.00 | 100.00 |

Ethanolic extracts tested at a concentration of 200 mg $L^{-1}$, Niclosamide at a concentration of 1 mg $L^{-1}$. Fractions tested at a concentration of 50 mg $L^{-1}$, Niclosamide at a concentration of 1 mg $L^{-1}$.

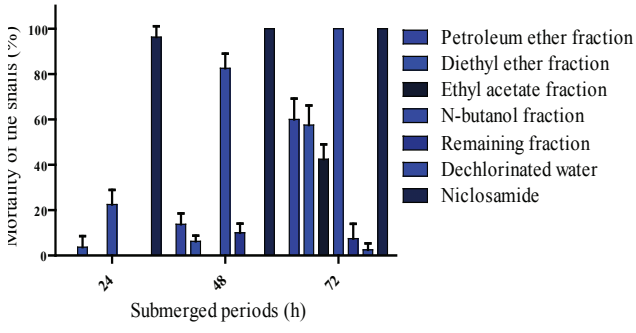


**Figure 1.** Different fractions of *Buddleja lindleyana* leaf against *Oncomelania hupensis* (n=4)

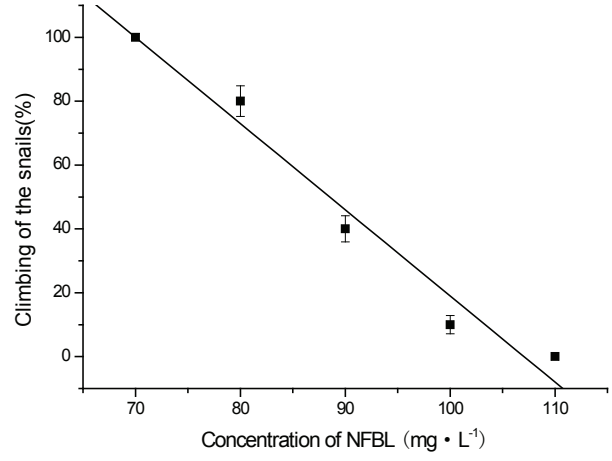


**Figure 2.** Concentration dependent between NFBL and climbing of the snails (n=4).

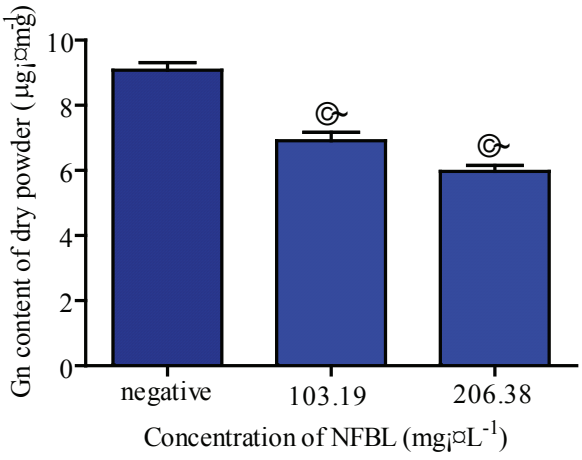


**Figure 3.** Gn content of soft tissues of snails treated by NFBL (n=4).

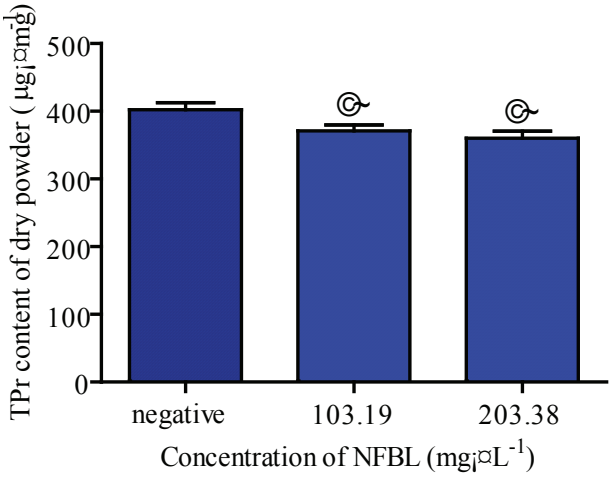


**Figure 4.** TPr content of soft tissues of snails treated by NFBL (n=4).

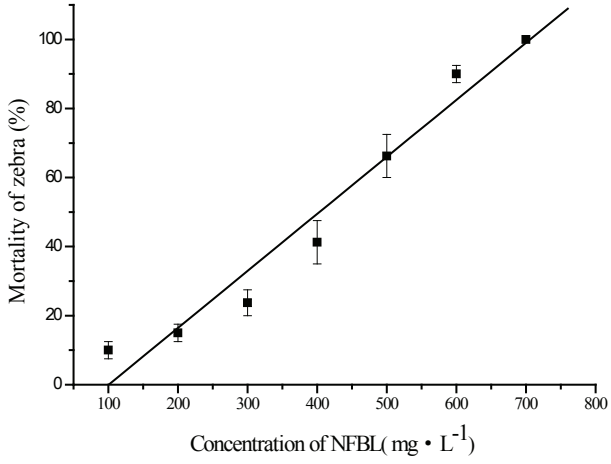


**Figure 5.** Concentration dependent between NFBL and mortality of zebrafish (n=4).

**Table 2.** The mortality of snails treated by NFBL (n=4) (M±SD).

| Concentration (mg $L^{-1}$) | Mortality (%) with different time | | |
|---|---|---|---|
| | 24 h | 48 h | 72 h |
| 15 | | 3.75±2.50 | 28.33±4.08 |
| 20 | | 7.50±9.57 | 51.67±9.13 |
| 25 | | 16.25±9.46 | 65.00±4.79 |
| 30 | | 26.25±9.46 | 78.33±4.79 |
| 35 | | 40.00±4.08 | 81.67±8.66 |
| 40 | | 51.25±4.79 | 88.33±4.08 |
| 45 | | 57.50±6.45 | 93.30±4.79 |
| 50 | 13.75±4.79 | 75.00±4.08 | 100.00 |
| 100 | 23.75±4.79 | | |
| 150 | 33.75±6.29 | | |
| 200 | 43.75±12.50 | | |
| 250 | 46.25±4.79 | | |
| 300 | 56.25±4.79 | | |
| LC50 | 257.98 | 39.91 | 19.99 |
| LC90 | 499.37 | 59.28 | 41.37 |

Mortality for dechlorinated tap water was 0 %, and the mortality for 1 mg $L^{-1}$ of Niclosamide was 100% for the test time.

## ACKNOWLEDGMENTS

Authors thank Mr. Long-gen Li and Bao-min Yang (Zhenjiang Center for Disease Control and Prevention, Jiangsu province, China) for providing snail and authentication. We also thank Dr. You-sheng Liang (Jiangsu Institute of Schistosome Diseases, Jiangsu Province, China) for useful suggestions.

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