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ORIGINAL PAPER

Screening of 42 medicinal plants for in vivo anthelmintic activity against *Dactylogyrus intermedius* (Monogenea) in goldfish (*Carassius auratus*)

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Abstract In the present study, methanol extracts of 42 traditional medicinal plants with potent anthelmintic activity against *Dactylogyrus intermedius* (Monogenea) in goldfish (*Carassius auratus*) were investigated. *Cinnamomum cassia*, *Lindera aggregata*, and *Pseudolarix kaempferi* exhibited 100% activity and were selected for further evaluation by applying five solvents (petroleum ether, chloroform, ethyl acetate, methanol, and water) for the extraction of the samples, followed by the in vivo bioassay. Among the extracts tested, water and methanol extracts of *C. cassia* showed the highest efficacies with EC_{50} values of 13.2 and 12.3 mg L^{-1}, showing 100% efficacy against *D. intermedius* at 30.0 and 40.0 mg L^{-1}, followed by methanol extract of *L. aggregata* which demonstrated 100% efficacy at 60.0 mg L^{-1} with EC_{50} value of 17.1 mg L^{-1} after 48 h of exposure. Methanol and ethyl acetate extract of *P. kaempferi*, which exhibited a 100% efficacy against *D. intermedius* at 60.0 and 50.0 mg L^{-1}, revealed similar activity with EC_{50} values of 23.5 and 23.3 mg L^{-1}, respectively. Acute toxicity of these active extracts was investigated on goldfish for 48 h and the corresponding median lethal concentrations (LC_{50}) of 56.9, 31.3, 88.7, 168.2, and 165.7 mg L^{-1}, respectively. These findings indicated that these extracts of the three plants can be developed as preferred natural antiparasitic agents for the treatment of *D. intermedius*.

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Introduction

China is a great fishery nation with a total yield of 51 million tons in 2005, which accounts for one-third of the total yield of the world, and was ranked number one in the world for the last 15 years (Zhou and Chen 2010). With the development of aquaculture industry, it has been overwhelmed with its share of diseases and problems caused by viruses, bacteria, fungi, parasites, and other undiagnosed and emerging pathogens (Bondad-Reantaso et al. 2005). *Dactylogyrus* spp., belonging to the family of Monogenea, are common ectoparasites living on the gills of freshwater fish and represent the largest group of metazoan fish parasites and major importance in the pathology of fishes (Woo et al. 2002). They have no intermediate hosts in their life cycle. The life cycle of *D. intermedius* comprises obligate adult stage, fertilized egg, and free-swimming larvae stage. The fertilized eggs develop into free-swimming ciliated larvae in the water column; the ciliated larvae are then carried to hosts by water currents as well as by their own ciliated movement (Klinger and Floyd 2002). *D. intermedius* attach to the gills, causing gill inflammation, excessive mucous secretions, accelerated respiration, and mixed infections with other parasites and secondary bacterial infections. Therefore, *D. intermedius* can cause serious damage to the host, such as loss of appetite, lowered growth performance, and high mortalities, which would result in great economic losses in aquaculture (Dove and Ernst 1998; Woo et al. 2002; Reed et al. 2009).

Chemical anthelmintics such as praziquantel, toltrazuril, and mebendazole have been used for decades throughout the world to minimize the losses caused the *Dactylogyrus*

infection (Schmahl and Mehlhorn 1985; Schmahl et al. 1988; Goven and Amend 1982). However, the frequent use of these chemical-based agents caused serious drawbacks such as environmental contamination, toxicity to the host, and even contamination of fish products with drug residues (Goven et al. 1980; Klinger and Floyd 2002), which prompted an urgent need for alternative therapy, including natural products from medicinal plants.

In recent years, there have been increasing interests in utilizing traditional medicinal plants for the control of parasitic infection. Several studies proved that different plant extracts have significant killing effects in vitro and in vivo on nematodes, cestodes, and trematodes (Mehlhorn et al. 2011; Klimpel et al. 2011; Abdel-Ghaffar et al. 2011). Hossain et al. (2011) evaluated the anthelmintic activity of the methanol extract of *Dregea volubilis* leaves against *Paramphistomum explanatum* and observed its effect through SEM study. The current work in our laboratory is focused on screening medicinal plants with promising anthelmintic activity and isolating groups of compounds/pure compounds responsible for the activity. We have previously reported that crude extracts of several traditional medicinal plants, such as *Arctium lappa* L., *Dioscorea zingiberensis* C. H. Wright, *Paris polyphylla*, *Angelica pubescens*, *Dryopteris crassirhizoma*, and *Cimicifuga foetida* L. (Wang et al. 2009a, 2010a, b; Liu et al. 2010; Lu et al. 2011; Wu et al. 2011) and some bioactive compounds, including arctigenin, trillin, gracillin, and dioscin (Wang et al. 2009a, b, 2010b) can effectively control the *D. intermedius* infection in goldfish (*Carassius auratus*). This study screened 42 kinds of medicinal plants for anthelmintic activity against *D. intermedius* in goldfish (*C. auratus*).

Materials and methods

Infected goldfish preparation

One-year-old goldfish (mean weight 4.2±0.5 g) without any record of foregone infestation with parasites were collected from a Changxing fish farm (Xian yang city, Shaanxi province, China). Then the stock was acclimatized in glass aquarium containing 180 L groundwater at 25±1°C (controlled by automatic aquarium heater) with aeration for 7 days and was fed with commercial pelleted goldfish diet at 2% of body weight. One week later, all the fish were cohabitated with the ones infected with *D. intermedius* which were reserved in our laboratory. The parasitized procedure was described in our previous study (Wang et al. 2008). Three weeks later, ten fish were randomly sampled and killed by spinal severance, and eight gill filaments of each fish were biopsied to determine the adult *D. intermedius* infestation level and intensity under a light microscope (Olympus BX41, Tokyo, Japan) at 10×4 magnification. Fish were chosen for the assays when the infection rate was 100% and the mean number of the parasite on gills was 40–50 per fish.

Collection of plant materials

The plant materials from each of the selected species (Table 1) were collected in August 2011 and identified by Prof. X.P. Song in Northwest A&F University (Shaanxi, China). The voucher specimens have been deposited at the Herbarium of the College of Life Science, Northwest A&F University, China. After oven-dried at 45°C for 48 h, the materials were crushed and reduced to fine powder using a strainer (30–40 mesh) manually with a disintegrator. The powdered samples were freeze-dried at −45°C to ensure complete removal of water.

The extraction of screened plants

The dry powder (50.0 g) of 42 kinds of plants was extracted with methanol (500 mL three times) for 48 h. In order to get more or less solidified crude extracts, the methanol filtrates were separately filtered and evaporated under reduced pressure in a vacuum rotary evaporator (R-201, Shanghai Shenshen) until the solvents completely evaporated. The resulting extracts of different plants were dissolved in dimethyl sulfoxide (DMSO) and diluted with distilled water to obtain 0.6 gmL^{-1} (sample/solvent) of stocking solutions, which were used for the preparations of the desired concentrations for anthelmintic efficacy assay.

The extraction of anthelmintic plants

Three plant materials (*C. cassia*, *L. aggregata*, and *P. kaempferi*) which have 100% anthelmintic efficacy were selected from 42 kinds of plants. Each plant material (50.0 g) was extracted with petroleum ether, chloroform, ethyl acetate, methanol, and water for 48 h for complete extraction, and the process was repeated three times. The ratio of sample to solvent was 1:10 (m/v). All the extracts were filtered, combined, and evaporated under reduced pressure in a vacuum rotary evaporator (R-201, Shanghai Shenshen). The resulting extracts of different plants were dissolved in dimethyl sulfoxide (DMSO) and diluted with distilled water to obtain 0.6 gmL^{-1} (sample/solvent) of stocking solutions, which were used in the further assay.

In vivo bioassays

Tests were conducted in each glass tank of 5-L capacity, filled with 2 L aerated groundwater, each containing

Table 1 Plants used in this study, their part used, the best anthelmintic efficacy, concentration of the best anthelmintic efficacy, and concentration with goldfish died against *Dactylogyrus intermedius* (Monogenea) in goldfish (*Carassius auratus*)

Species	Plant part used	The best anthelmintic efficacy (%)	Concentration of the best anthelmintic efficacy (mg/L)	Concentration with goldfish died (mg/L)
Cinnamomum cassia Presl.	Tree bark	100	200	300
Lindera aggregata (Sims) Kosterm.	Roots	100	40	60
Pseudolarix kaempferi Gord.	Tree bark	100	100	120
Aquilaria sinensis (Lour.) Gilg.	Tree	89	40	40
Stephania tetrandra S.Moore	Roots	80	800	800
Coix lacryma-jobi L. var. *ma-yuen* (Roman.) Stapf	Kernal	75	600	600
Foeniculum vulgare Mill.	Fruits	72	600	600
Dictamnus dasycarpus Turcz.	Bark of rhizome	64	20	20
Cynanchum atratum Bge.	Roots	60	500	500
Magnolia denudata Desv.	Alabastrum	53	30	30
Ligusticum chuanxiong Hort.	Rhizome	50	100	100
Ligustrum lucidum Ait.	Fruits	50	600	600
Allium tuberosum Rottl.	Seeds	47	300	300
Menispermum dauricum DC.	Roots	44	300	300
Lonicera japonica Thunb.	Alabastrum	41	600	600
Magnolia officinalis Rehd. et Wils.	Bark	40	100	100
Astragalus complanatus R.Brown.	Seeds	39	200	200
Gentiana manshurica Kitag.	Rhizome	35	600	600
Cirsium japonicum DC.	Herbs and roots	28	90	90
Rehmannia glutinosa Libosch.	Roots, rhizome	20	600	600
Acorus tatarinowii Schott	Rhizome	0	–	1000
Alisma orientalis (Sam.) Juzep.	Rhizome	0	–	600
Angelica sinensis (Oliv.) Diels	Roots	0	–	300
Asarum heterotropoides Fr. Schmidt var. *mandshuricum* (Maxim.) Kitag.	Herbs	0	–	2
Bolbostemma paniculatum (Maxim.) Franquet	Rhizome	0	–	100
Corydalis yanhusuo W. T. Wang	Rhizome	0	–	100
Daphne giraldii Nitsch.	Bark of rhizome	0	–	20
Eugenia caryophllata Thunb.	Alabastrum	0	–	50
Glehnia littoralis Fr. Schmidt ex Miq.	Roots	0	–	400
Lobelia chinensis Lour.	Herbs	0	–	1000
Morinda officinalis How	Roots	0	–	400
Plantago asiatica L.	Fruits	0	–	400
Polygonatum sibiricum Red.	Rhizome	0	–	>1000
Polygonum multiflorum Thunb.	Rhizome	0	–	400
Pueraria lobata (Willd.) Ohwi.	Roots	0	–	>1000
Raphanus sativus L.	Seeds	0	–	>1000
Rubia cordifolia L.	Roots	0	–	600
Schisandra chinensis (Turcz.) Baill.	Fruits	0	–	40
Senecio scandens Buch.-Ham.	Herbs	0	–	500
Siegesbeckia orientalis L.	Aerial parts	0	–	400
Speranskia tuberculata (Bunge) Baill.	Herbs	0	–	1000
Typhonium giganteum Engl.	Rhizome	0	–	160

– Not analyzed

samples and five previously infected fish. The water pH ranged from 7.0 to 7.5, and dissolved oxygen was between 6.2 and 7.8 gmL^{-1} (72–85% saturation); the water temperature was constant at 24±1°C. Initial tests were conducted

to get a moderate concentration range in order to avoid the mortality of fish at high concentrations.

For the extracts of screened plants

The designed concentration gradients of each extract were added; the final concentrations in the test solution were 100, 200, 300, 400, 500, and 600 mg L^{-1}. Negative control groups containing no plant extract were set up under the same conditions as the test groups. To discard the possible effects of DMSO on the parasites, another control, containing the highest percentage of DMSO, was included.

For the extracts of anthelmintic plants

The crude extracts of *C. cassia*, *L. aggregata*, and *P. kaempferi* were conducted at a different series of concentrations based on the initial tests, respectively, and the negative control groups containing no plant extract were set up under the same conditions as the test groups. The DMSO control was also included.

All the experiments were conducted twice. During the experiments, no food was offered to the fish. The death of fish was recorded when the opercula movement and tail beat stopped and the fish no longer responded to mechanical stimulus. To avoid the deterioration of the water quality, the observed dead fish were removed from the water in time. Forty-eight hours later, the surviving fish in all the treatments were killed by spinal severance and biopsied under a light microscope at 4×10 magnification (Fig. 1). The anthelmintic efficacy of each treatment and the negative control group was calculated according to the following formula:

$$\mathrm{AE} = (B - T)/B \times 100$$

where AE is anthelmintic efficacy, B is average number of surviving *D. intermedius* in the negative control, and T is average number of surviving *D. intermedius* in the treatment groups.

Acute toxicity test

Acute toxicity tests were performed in a 5-L capacity plastic pot with 2 L of the test solution water and ten healthy goldfish. Control groups were set under the same test conditions without extracts. Another control group containing the highest percentage of DMSO was also included. The experiments were performed twice at 24±1°C. The death of fish was recorded when the opercula movement and tail beat stopped and the fish no longer responded to mechanical stimulus. To avoid the deterioration of the water quality, the observed dead fish were removed from the water in time.

Statistical analysis

The homogeneity of the replicates of the samples was checked by the Mann–Whitney U test. Probit analysis was used for calculating the median lethal concentration (LC_{50}, LC_{90}) and median effective concentration (EC_{50}, EC_{90}) at the 95% confidence interval with upper confidence limit and lower confidence limit (Finney 1971).

Result

The anthelmintic efficacies against *D. intermedius* (Monogenea) of selected plants were evaluated, and the results are shown in Table 1. Among the screened plants, *P. kaempferi*, *L. aggregata*, and *C. cassia* were found to have 100% anthelmintic efficacy at 60.0, 120.0, and 300.0 mg L^{-1}. The solvent (DMSO) acting as a control showed no anthelmintic activity when treated at the highest concentration.

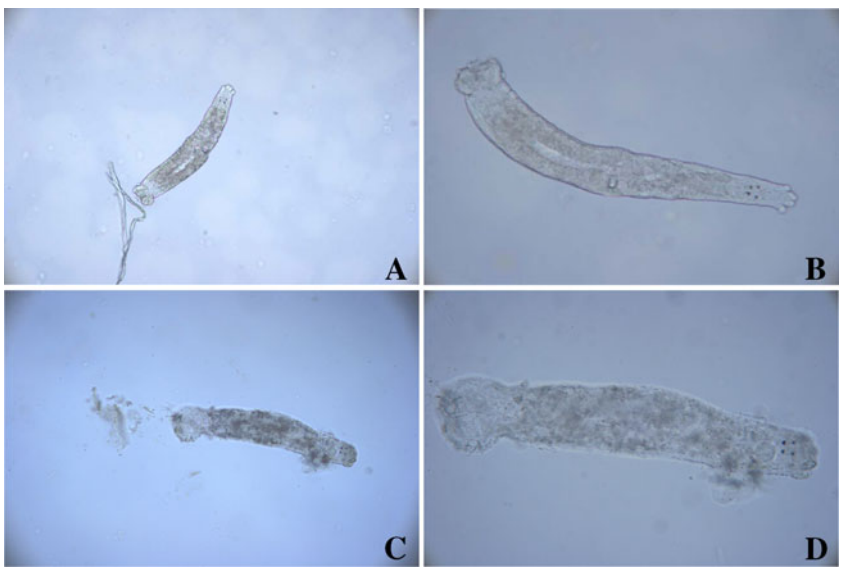

Fig. 1 Micrographs of untreated (**a**, **b**) and treated (**c**, **d**) *D. intermedius*. **a**, **b** Microscopic slide with live *D. intermedius* detached from the gills under a light microscope at 20×10 and 40×10 magnification. **c**, **d** Microscopic slide with dead *D. intermedius* detached from the gills after treated with the methanol extract of *C. cassia* at 20×10 and 40×10 magnification

The anthelmintic efficacies of different extracts of *C. cassia*, *L. aggregata*, and *P. kaempferi* are depicted in Fig. 2, and the EC_{50} and EC_{90} values are shown in Fig. 3. The methanol extract of *C. cassia* was found to be the most effective one with EC_{50} and EC_{90} values of 12.3 and 32.1 mg L^{-1}, respectively. After exposure for 48 h, it exhibited a 100% efficacy against *D. intermedius* at 30.0 mg L^{-1}. High anthelmintic activity against *D. intermedius* was also observed in the water and chloroform extracts with EC_{50} and EC_{90} values of 13.2, 29.4, 39.5, and 93.6 mg L^{-1}, respectively. The ethyl acetate and petroleum ether extracts, however, exhibited weak activity with the maximum anthelmintic efficacy of 6.1% at 40.0 mg/L and 31.1% at 100.0 mg L^{-1} with no fish died.

In the case of *L. aggregata*, the methanol extracts were observed to be the most effective with EC_{50} and EC_{90} values of 23.5 and 37.7 mg L^{-1} after 48 h of treatment, respectively. The methanol extracts exhibited a 100% anthelmintic efficacy against *D. intermedius* at 60.0 mg L^{-1}. The extracts of water and ethyl acetate also showed high anthelmintic activity, with EC_{50} and EC_{90} values of 366.0, 632.7, 109.6, and 156.0 mg L^{-1}, respectively. However, fish mortality occurred when the concentration reached 600.0 mg L^{-1} for water and 200 mg L^{-1} for ethyl acetate, followed by the chloroform and

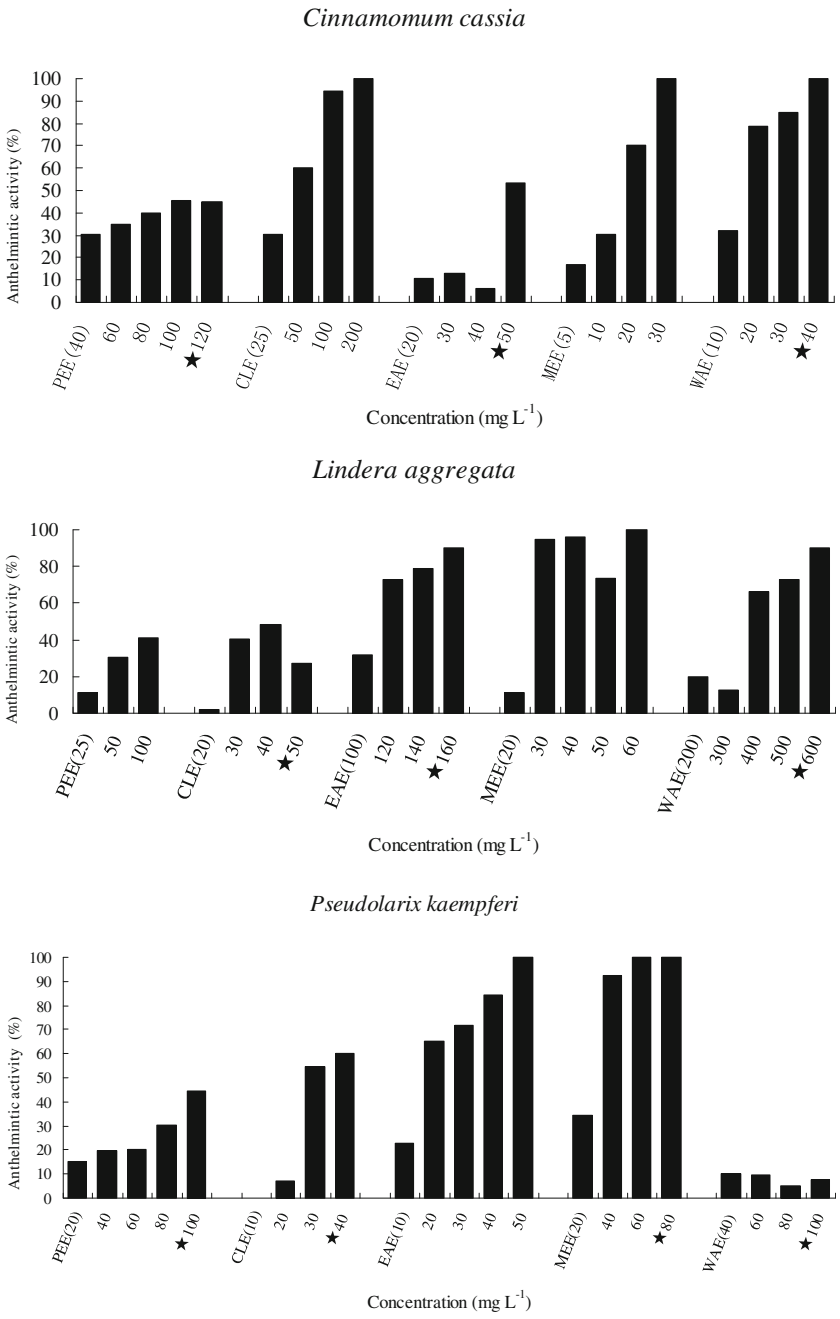

Fig. 2 Anthelmintic efficacy of different extracts of *Cinnamomum cassia*, *Lindera aggregata*, and *Pseudolarix kaempferi* against *Dactylogyrus intermedius* after 48 h. *PEE* petroleum ether extract, *CLE* chloroform extract, *EAE* ethyl acetate extract, *MEE* methanol extract, *WAE* water extract. *Star* indicates when fish mortality first occurred

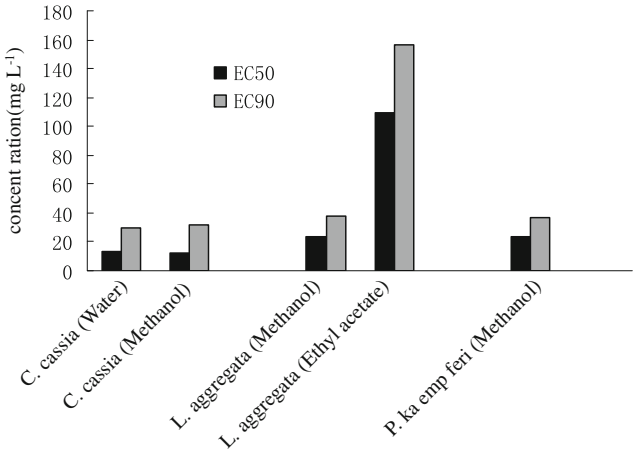

Fig. 3 Anthelmintic efficacy of extracts from *Cinnamomum cassia*, *Lindera aggregata*, and *Pseudolarix kaempferi* against *Dactylogyrus intermedius* after 48 h of exposure

the petroleum ether extracts, which exhibited anthelmintic efficacies of 27.27% and 16.17% both at 50.0 mg L^{-1}.

As for *P. kaempferi*, both methanol and ethyl acetate extracts displayed the optimal anthelmintic activity with 100% efficacy at the dose of 60.0 mg L^{-1}. EC_{50} and EC_{90} values were 23.3 and 37.0 mg L^{-1} for methanol extract, and 17.1 and 42.8 mg L^{-1} for ethyl acetate extract. The remaining other plant extracts were found to exhibit weak activity with the highest anthelmintic efficacy of 0% for water, 54.6% for chloroform, and 44.4% for petroleum ether, respectively.

The results of acute toxicity assay for methanol and water extracts of *C. cassia*, the methanol extracts of *L. aggregata*, and the methanol and ethyl acetate extracts of *P. kaempferi* are shown in Table 2. The 48-h LC_{50} values of methanol extracts of *C. cassia*, *L. aggregata*, and *P. kaempferi* were 31.3, 165.7, and 88.7 mg L^{-1}; the water extract of *C. cassia* was 56.9 mg L^{-1}, and the ethyl acetate extract of *P. kaempferi* was 168.2 mg L^{-1}.

Discussion

The monogenean trematodes are mostly parasitic in the gills and body surface of freshwater fish and treated as a serious pest in the aquaculture industry (Oliver 1977). *Dactylogyrus*, a main genus of monogeneans, is a common parasite in fish diseases. Traditionally, a number of chemotherapeutic agents have been used to deal with severe problems caused by *Dactylogyrus*. However, because of their side effects, such as accumulation of drugs in tissues, development of drug resistance, and the potential deleterious effects on the environment and the human consumers, the use of these chemicals are not recommended anymore. Screening of medicinal plants and application of their extracts to control monogenean parasites could offer possible alternatives that may be both sustainable and environmentally acceptable. For this reason, the plant-based products have been extensively studied to control *Dactylogyrus* infection as compared with the chemicals. In the present study, 42 medicinal plants were evaluated for the in vivo anthelmintic activity against *D. intermedius* (Monogenea) in goldfish (*C. auratus*). Three plants, namely *C. cassia*, *L. aggregata*, and *P. kaempferi*, were found to produce a 100% parasite elimination rate at low concentration. As far as we know, this is the first report on the anthelmintic activity of *C. cassia*, *L. aggregata*, and *P. kaempferi*.

Among the three potent plants, extracts from *C. cassia* were found to exhibit strongest efficacy with the lowest EC_{50} and EC_{90}. The dried stem bark of *C. cassia* is a popular natural spice and a commonly used herb in traditional Chinese medicine. Shan et al. (1999) found that the water extract of *C. cassia* enhanced Ig production by B cells, IL-1 production by monocytes, and cytotoxic T-lymphocyte activity against allogeneic tumor cells. The ethanol extract of *C. cassia* exhibited the strongest antioxidant action not only in the rat homogenate model system but also in the cytochrome test. Meanwhile, the ethanol extract also displayed anti-superoxide formation activity and is treated as an excellent xanthine oxidase inhibitor (Lin et al. 2003). The biologically active constituents of *C. cassia* are cinnamaldehyde, cinnamon oil, eugenol, salicylaldehyde and trans-cinnamic acid. Ooi et al. (2006) pointed out that the hydro-distilled Chinese cinnamon oil and pure cinnamaldehyde of *C. cassia* were equally effective in inhibiting the growth of various isolates of bacteria including Gram-

Table 2 Forty-eight-hour acute toxicity of water and methanol extracts from *Cinnamomum cassia*, methanol extract from *Lindera aggregata*, and methanol and ethyl acetate extracts from *Pseudolarix kaempferi* against goldfish

Plants	Extraction solvent	LC_{50} (mg/L) (95% CL)	LC_{90} (mg/L) (95% CL)	χ^2
Cinnamomum cassia	water	56.9 (16.0–89.6)	88.1 (67.5–267.4)	1.176
	Methanol	31.3 (22.8–38.7)	40.6 (34.7–64.6)	1.309
Lindera aggregata	Methanol	165.7 (148.2–180.6)	183.4 (171.6–252.4)	0.347
Pseudolarix kaempferi	Methanol	88.7 (81.2–98.3)	98.1 (91.8–130.3)	1.281
	Ethyl acetate	168.2 (151.1–191.3)	199.9 (180.5–303.5)	2.127

LC_{50} lethal concentration 50%, *LC_{90}* lethal concentration 90%, *95% CL* 95% confidence limit

positive and Gram-negative, and fungi including yeasts and dermatophytes. The eugenol and salicylaldehyde revealed strong insecticidal activity, whereas trans-cinnamic acid revealed moderate activity (Park et al. 2000). Considering the major bioactive constituents of *C. cassia*, some of the substances mentioned earlier may contribute to the efficacy of *C. cassia* independently or jointly.

Radix Linderae, the root tuber of *L. aggregata*, is a traditional herbal medicine in both China (Wu-yao) and Japan (Uyaku) for treating several diseases including chest and abdomen pain, indigestion, regurgitation, cold hernia, and frequent urination (Jiangsu New Medical College 1979; The Editorial Committee of the Administration Bureau of Traditional Chinese Medicine 1999). The extracts of *Radix Linderae* have been reported to possess anti-inflammatory, analgesic, and antimicrobial properties (Chou et al. 1999). Study on *Radix Linderae* revealed that it contained alkaloids, volatile oils, and sesquiterpene esters. Luo et al. (2009) suggested that the total alkaloids from *Radix Linderae* exhibited inhibitory effects on the production of inflammatory mediators from macrophages via blocking NF-κB and MAPKs signaling pathways. In the case of *Radix Linderae* essential oil, it is useful to improve the immunity activities and prevent the occurrence of decubitus in aged people (Liang 2011). These findings provide a plausible explanation for the high LC_{50} and LC_{90} of the methanol extract of *L. aggregata*. As for *P. kaempferi*, it is a kind of indigenous plant in the east of China. Its root bark known as "Tu Jin Pi" is used in traditional Chinese medicine for the treatment of skin diseases caused by microbial infection. The diterpenoids, pseudolaric acid A, and pseudolaric acid B were found to be the antifungal component of this plant (Li et al. 1995; Yang et al. 2003). Zhang et al. (1990) observed that pseudolaric acid has a partial antifertility effect when it was injected ig 20 mg/kg daily to hamsters (female) for 4 days before mating. Pseudolarolides B, which belongs to triterpene lactone, showed potent cytotoxicity against three human cancer cell lines, KB (nasopharyngeal), A-549 (lung), and HCT-8 (colon), and a murine leukemia cell line (P-388) with ED_{50} values of 0.49, 0.67, 0.73, and 0.79 μg/mL, respectively (Chen et al. 1993).Two triterpenoids, isopseudolarifuroic acids A and B, exhibited significant cytotoxic activities against several tumor cell lines (Yang and Yue 2001). The high cytotoxic potency of triterpenoids and triterpene lactone might be involved in the eradication of the parasites. Although there are no attempts to identify the anthelmintic compound(s) in these two plants, some of the substances mentioned earlier are believed to contribute jointly or independently to the inhibition activity against *D. intermedius*.

Among the other 39 kinds of plants screened, a large portion exhibited high anthelmintic activity against *D. intermedius* but less than 100%. These may be the result of that the ingredients which are responsible for the anthelmintic activity are totally different from the ones which are toxic to the fish. Comparing the EC_{50} and EC_{90} of *C. cassia* and *P. kaempferi*, the methanol extract of *C. cassia* showed a close EC_{50} and EC_{90} with the water extract. Meanwhile, the similar phenomenon was also found in methanol and ethyl acetate extracts of *L. aggregata*. The results of acute toxicity assay for the extracts of *C. cassia*, *L.* aggregata, and *P. kaempferi* indicated that these extracts were safe to goldfish. The 48-h LC_{50} values of these extracts were higher than the corresponding EC_{50}. For example, for the ethyl acetate extract of *P. kaempferi*, the toxic dose (LC_{50}=168.2 mg L^{-1}) is about ten times the effective one (EC_{50}=17.1 mg L^{-1}).These results enhance the possibility of the development and use of commercial products containing this material.

In summary, the extracts of *C. cassia*, *L. aggregata*, and *P. kaempferi* have the potential for the development of novel therapy for the treatment against *D. intermedius* infection. However, more investigations such as pharmacological evaluations before clinical trials, assessment of ecological risk posed by practical usage, and their detailed mechanism of anthelmintic (*D. intermedius*) activity must be performed. Further bioassay-guided isolation and purification of compound(s) responsible for the observed anthelmintic efficacy are in progress.

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