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ARTICLE *in* PARASITOLOGY RESEARCH · DECEMBER 2010

Impact Factor: 2.1 · DOI: 10.1007/s00436-010-2211-5 · Source: PubMed

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Parasitol Res (2011) 108:1557–1563
DOI 10.1007/s00436-010-2211-5

ORIGINAL PAPER

In vivo evaluation of anthelmintic potential of medicinal plant extracts against *Dactylogyrus intermedius* (Monogenea) in goldfish (*Carassius auratus*)

Zong-Fan Wu · Bin Zhu · Yong Wang · Cheng Lu · Gao-Xue Wang

Received: 19 November 2010 / Accepted: 30 November 2010 / Published online: 14 December 2010

Abstract In the present study, an attempt has been made to petroleum ether, chloroform, ethyl acetate, methanol, and water extracts of *Prunus amygdalus* Batsch seeds (*Semen amygdali*), *Cimicifuga foetida* L. rhizomes (*Rhizoma Cimicifugae*), *Peucedanum decursivum* (Miq.) *Maxim* roots (*Radix Peucedani*), *Momordica cochinchinensis* Spreng. seeds (*Semen Momordicae*), and *Bupleurum chinense* DC. roots (*Radix Bupleuri chinensis*) for their in vivo anthelmintic activity against monogenean *Dactylogyrus intermedius* in goldfish (*Carassius auratus*). The results showed that the efficacies of methanol, chloroform, and ethyl acetate extracts from *R. Bupleuri chinensis* were found to be, in this order, more effective than others with the 48 h-EC_{50} and EC_{90} values of 3.5 and 6.9, 6.0 and 8.4, 7.4 and 11.2 mg/L, respectively, followed by ethyl acetate extract of *R. cimicifugae* and chloroform extract of *R. peucedani* with EC_{50} 189.2 and 240.4 mg/L. The promising methanol, chloroform, and ethyl acetate extracts from *R. Bupleuri chinensis* were subjected to acute toxicity tests for the evaluation of their safety to the host. After 48-h exposure, the mortalities of goldfish were recorded, and the established LC_{50} values were 10.1-, 4.2-, and 8.4-fold higher than the corresponding EC_{50}. These results indicated that the three extracts from *R. Bupleuri chinensis* exhibit potential to be used as preferred natural antiparasitics for the control of the *D. intermedius*, especially for the methanolic one.

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Introduction

Nowadays, aquaculture is considered as one of the fastest-expanding industries worldwide, and nowhere in the world is more apparent than in Asia, a region that currently accounts for around 94% of the total world production. China, as one of the leading aquaculture nations in the world, alone accounts for approximately 71% of the total global aquaculture production (Sapkota et al. 2008). However, the extensive commercial production was frequently accompanied by occurrence of various infectious diseases caused by bacterial, viral, and parasites, which resulted in great losses to the aquaculture and impeded further development of this industry (Bondad-Reantaso et al. 2005).

Dactylogyrus spp., belonging to the family of Monogenea, are common ectoparasites living on the gills of freshwater fish and present the largest group of metazoan fish parasites and major importance in the pathology of fishes (Woo et al. 2002). These parasites are egg layers with two to four eyespots and one pair of large anchor hooks, and have a direct life cycle without intermediate host (Wang et al. 2010a). Their life cycle comprises an obligate adult stage, fertilized egg, and free-swimming larvae stage. The fertilized eggs are released into water column, and then developed into free-swimming ciliated larvae, followed by attaching to the gills of new hosts via water currents and their own ciliated movement (Klinger and Floyd 2002). The fish, heavily infected with *Dactylogyrus*, would not only show the swollen and pale gills, excessive mucous secretions and increasing respiration rate, but also be more easily subjected to mixed infections with other parasites and secondary bacterial and fungal infections, resulting in a serious damage to the host, such as loss of appetite, lowered growth performance, and high mortalities (Dove and Ernst 1998; Woo et al. 2002; Reed et al. 2009).

Existing methods of *Dactylogyrus* control rely heavily on chemical anthelmintic agents. The most effective treatment has been achieved by use of formalin when administrated as a prolonged bath at low dose or short-term bath at higher dose (Thoney and Hargis 1991). However, the use of formalin has been discouraged, because this is considered to bear risks for humans and environment. For example, it can lead to the eradication of algae in the pond and subsequent depletion of dissolved oxygen (Ekanem et al. 2004a). Other chemicals, including toltrazuril, praziquantel, and mebendazole, have also been evaluated for chemotherapy of *Dactylogyrus* under laboratory conditions and also played a pivotal role in the treatment of *Dactylogyrus* infestation in aquaculture practice (Schmahl and Mehlhorn 1985; Schmahl et al. 1988; Treves-Brown 1999). However, application of these chemical-based agents has had limited efficacy due to the raised drug-resistance caused by the frequent use of these drugs and is often accompanied by serious drawbacks, such as the environmental contamination, toxicity to the host, and even contamination of fish products with drug residues, which have stimulated the search for new control strategies (Goven et al. 1980; Klinger and Floyd 2002).

Evaluation of the activities of medicinal plants claimed for anthelmintic property is getting attention these days (Tóro et al. 2003; Ekanem et al. 2004a, 2004b; Chansue 2007). Screening and proper evaluation of the claimed medicinal plants could offer the possible alternatives that may both be sustainable and environmentally acceptable (Eguale et al. 2007). 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. (Wang et al. 2009), *Dioscorea zingiberensis* C. H. Wright (Wang et al. 2010a) and *Paris polyphylla* (Wang et al. 2010b) can effectively control the *Dactylogyrus intermedius* infection in goldfish (*Carassius auratus*), and some bioactive compounds have further been isolated, including arctigenin, gracillin, dioscin, and so on. Nevertheless, there is still little available information on the use of medical plants for the treatment of *Dactylogyrus* diseases in fish. An attempt was, therefore, made under the present work to exploit the different solvent extracts of *Semen amygdali* (seeds of *Prunus amygdalus* Batsch), *Rhizoma Cimicifugae* (rhizomes of *Cimicifuga foetida* L.), *Radix Peucedani* (roots of *Peucedanum decursivum* (Miq.) *Maxim*), *Semen Momordicae* (seeds of *Momordica cochinchinensis* Spreng.), and *Radix Bupleuri chinensis* (roots of *Bupleurum chinense* DC.) for their anthelmintic activity against *Dactylogyrus intermedius* in goldfish. To the best of our knowledge, this is the first report on the anthelmintic potential of the crude extracts of the selected plants.

Materials and methods

Parasites and hosts

Healthy goldfish, with no record of previous infestation with parasites (weight mean±SD, 3.6±0.7 g), were obtained from Shaanxi Fisheries Research Institute (Shaanxi, China) and maintained in a 180-L aerated glass aquarium with oxygen content higher than 85% saturation at 23±1°C. After a stress-free acclimatization process under laboratory conditions for 7 days, fish were periodically mixed into the ones infested with *D. intermedius* which were maintained in our laboratory. The infected fish were prepared following the method described in our previous work (Wang et al. 2008). Briefly, this procedure consisted of collection of eggs, hatching of eggs, developing into oncomiracidial larvae, and reinfection of healthy fish. After 3 weeks, ten fish were randomly selected, killed by spinal severance, and checked for the presence and intensity of parasites on the gills of infected fish under a light microscope (Olympus BX41, Tokyo, Japan) at 10×4 magnification. Fish were used for the in vivo anthelmintic efficacy assay when the infestation prevalence is 100%, and the mean number of the parasite on gills of fish is 40–50.

Collection of plant materials and preparation of extracts

Fresh plant materials from each of the selected species (see Table 1) were collected in 2009. The plants were

Table 1 Selected medicinal plants for anthelmintic evaluation

Plants	Family	Part used	Collection place
Cimicifuga foetida L.	Ranunculaceae	Rhizomes	Shaanxi, Shangluo, China (34°17′21″N, 109°35′40″E, 1340 m)
Peucedanum decursivum (Miq.) *Maxim*	Umbelliferae	Roots	Anhui, Ningguo, China (30°52′16″N, 118°42′50″E, 986 m)
Momordica cochinchinensis Spreng.	Cucurbitaceae	Seeds	Fujian, Shaxian, China (26°09′31″N, 117°50′53″E, 513 m)
Prunus amygdalus Batsch	Rosaceae	Seeds	Shaanxi, Yulin, China (37°26′34″N, 110°41′16″E, 1129 m)
Bupleurum chinense DC.	Umbelliferae	Roots	Shanxi, Ruicheng, China (34°23′05″N, 110°18′07″E, 693 m)

identified by Prof. X.L. He in Northwest A&F University (Shaanxi, China), and voucher specimens have been deposited at the Herbarium of the College of Life Sciences of the university. Plant materials were washed, cut into small pieces, and dried in sunlight or in an oven at 50°C maximum. Dried materials were crushed manually with a mortar and pestle and reduced to fine powder using a strainer (30–40 mesh). The powdered sample was freeze-dried at −54°C to ensure a complete removal of water. Five powdered dry samples of each plant material (20.0 g) were respectively 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*). Each extract was subsequently filtered and concentrated under reduced pressure in a vacuum rotary evaporator (R-201, Shanghai Shenzhen) until the solvents were completely evaporated to get more or less solidified crude extracts. The crude petroleum ether, chloroform, ethyl acetate, methanol, and water extracts of *S. amygdali*, *R. Cimicifugae*, *R. Peucedani*, *S, Momordicae*, and *R. Bupleuri chinensis*, respectively, were dissolved in 40 mL of dimethyl sulfoxide (DMSO) to get 0.5 g/mL (sample/solvent) of stocking solutions, which were used for the preparations of the desired concentrations for anthelmintic efficacy assay.

In vivo anthelmintic efficacy assay

The anthelmintic activities of five solvent extracts of *S. amygdali*, *R. cimicifugae*, *R. peucedani, S. momordicae* and *R. bupleuri chinensis* were assayed in 5-L plastic pot, containing 2 L of the working solutions and five infected goldfish at a different series of concentrations based on initial tests. The water pH ranged from 7.0 to 7.5, dissolved oxygen was between 6.5 and 7.7 mg/L, and the water temperature was constant at 23±1°C. Negative control groups with no extract were set up under the same experimental conditions, while mebendazole was used as the positive control with different series concentrations of 0.6, 1.0, 1.5, 2.0, and 2.5 mg/L. To discard the possible effects of DMSO on the parasites, another control, containing the highest percentage of DMSO, was also included. All treatment and control groups were conducted in triplicate. After 48 h, all the surviving goldfish in all the treatment and control groups were killed by a spinal severance for biopsy. The lamella branchialis were placed on glass slides, and the numbers of parasites on the gills were counted under a light microscope (Olympus BX41, Tokyo, Japan) at 4×10 magnification to determine the mean number of parasites per infected goldfish. The anthelmintic efficacies of each treatment and the positive control group were calculated according to the following formula (Wang et al. 2008):

$$AE(\%) = [B - T(P)]/B \times 100$$

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

Acute toxicity test

Based on the results of in vivo anthelmintic efficacy assay, the promising chloroform, ethyl acetate, and methanol extracts from *R. Bupleuri chinensis* were subjected to acute toxicity tests for the evaluation of their safety to the host. Tests were conducted in duplicate, using ten healthy goldfish in plastic pot with 5-L capacity, containing 2 L of filtered water at 23± 1°C. Control groups were set under the same test conditions without extracts. The death of fish were recorded when the opercula movement and tail beat stopped and the fish no longer responded to mechanical stimulus. During the experiments, the observed dead fish was removed in time from the water to avoid the deterioration of the water quality. Fish mortalities in the treatment and control groups were recorded after 48 h of exposure.

Statistical analysis

The homogeneity of the replicates of the samples was checked by the Mann–Whitney *U* test. Probit analysis (Finney 1971) was used for the calculation of EC_{50}, EC_{90}, LC_{50}, and LC_{90} at the 95% confidence level.

Results

In vivo anthelmintic efficacy

The anthelmintic efficacies with the EC_{50} and EC_{90} values of different solvent extracts of *R. Cimicifugae*, *R. Peucedani*, *S. Momordicae*, *S. amygdali*, and *R. Bupleuri chinensis* were depicted in Fig. 1 and Table 2, which indicated that the crude methanolic extract of *R. Bupleuri chinensis* was found to be most effective with EC_{50} value of 3.5 mg/L and EC_{90} value of 6.9 mg/L after exposure for 48 h, while it exhibited a 100% efficacy against *D. intermedius* at 10.0 mg/L. High anthelmintic activity against *D. intermedius* was also observed in the chloroform and ethyl acetate extracts from *R. Bupleuri chinensis* with EC_{50} and EC_{90} values of 6.0 and 8.4 mg/L for the chloroform extract and 7.4 and 11.2 mg/L for the ethyl acetate extract, respectively. In these cases, increasing concentrations of exposure to the extracts were strongly associated with increased removal of *D. intermedius*.

The petroleum ether and water extracts, however, exhibited weak activity with the maximum anthelmintic efficacy of 16.2% at 150.0 mg/L and 31.6% at 40 mg/L.

With respect to *R. Cimicifugae*, *R. Peucedani*, *S. Momordicae*, and *S. amygdali*, the ethyl acetate extract of *R. Cimicifugae* was observed to be the most effective one with the least EC_{50} and EC_{90} values of 189.2 and 402.0 mg/L after 48 h exposure. Similar anthelmintic efficacy was also exhibited by chloroform extract of *R. Peucedani*. However, in this case, fish mortality occurred at the highest concentration (350 mg/L), which lead to the maximum anthelmintic efficacy of 80.3%. As shown in Fig. 1 and Table 2, the remaining other plant extracts were found to exhibit weak activity with the larger EC_{50} and EC_{90} values or to be highly toxic to the host.

The DMSO, acted as control, showed no anthelmintic activity when treated at the highest concentration (less than 0.5%), and the calculated EC_{50} and EC_{90} values of the positive control, mebendazole, were 1.3 (95% CL, 1.0–1.5) and 3.7 (95% CL, 2.9–6.1) mg/L, respectively.

Acute toxicity

The results of acute toxicity assay for chloroform, ethyl acetate, and methanol extracts from *R. Bupleuri chinensis*

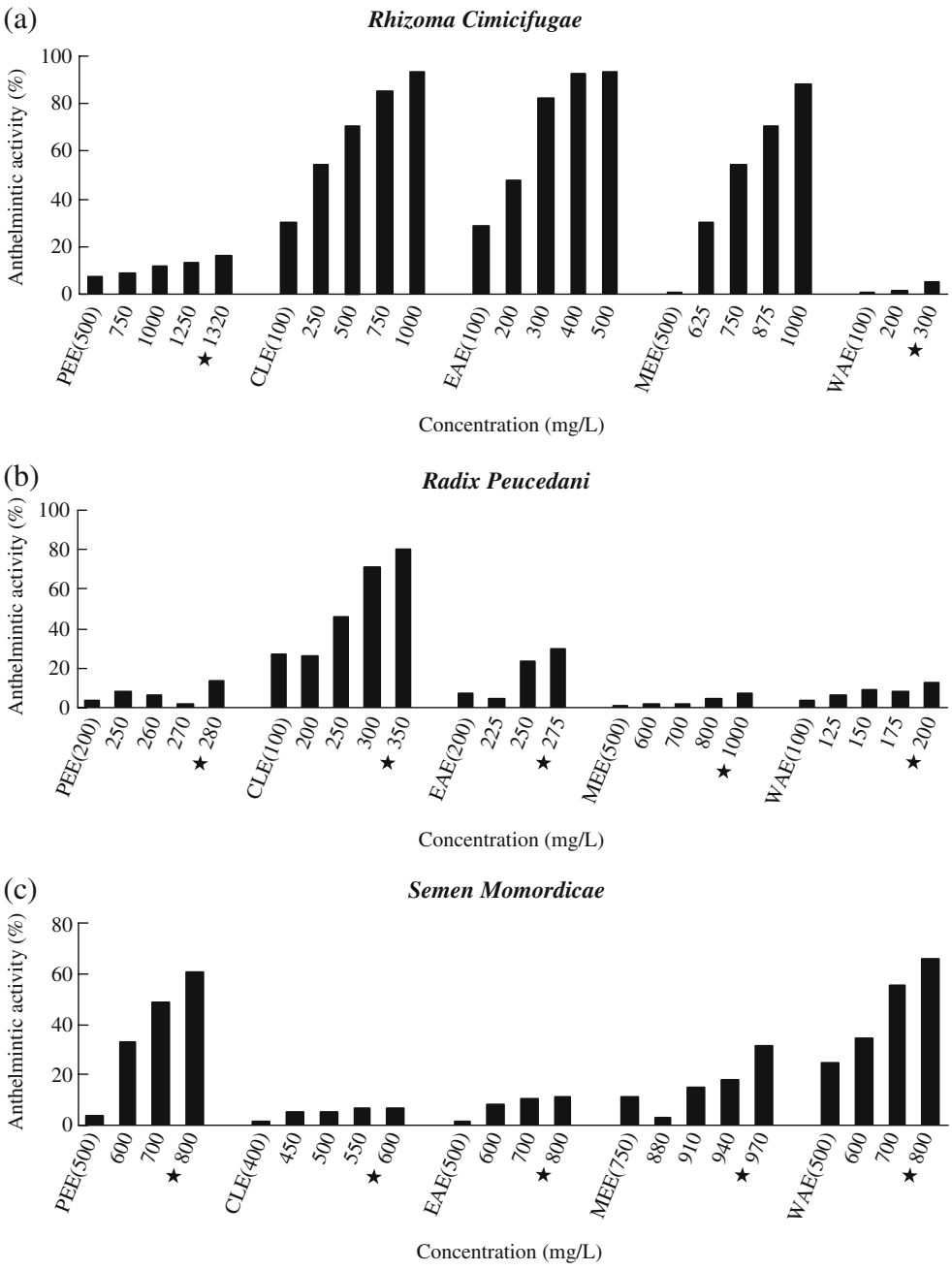
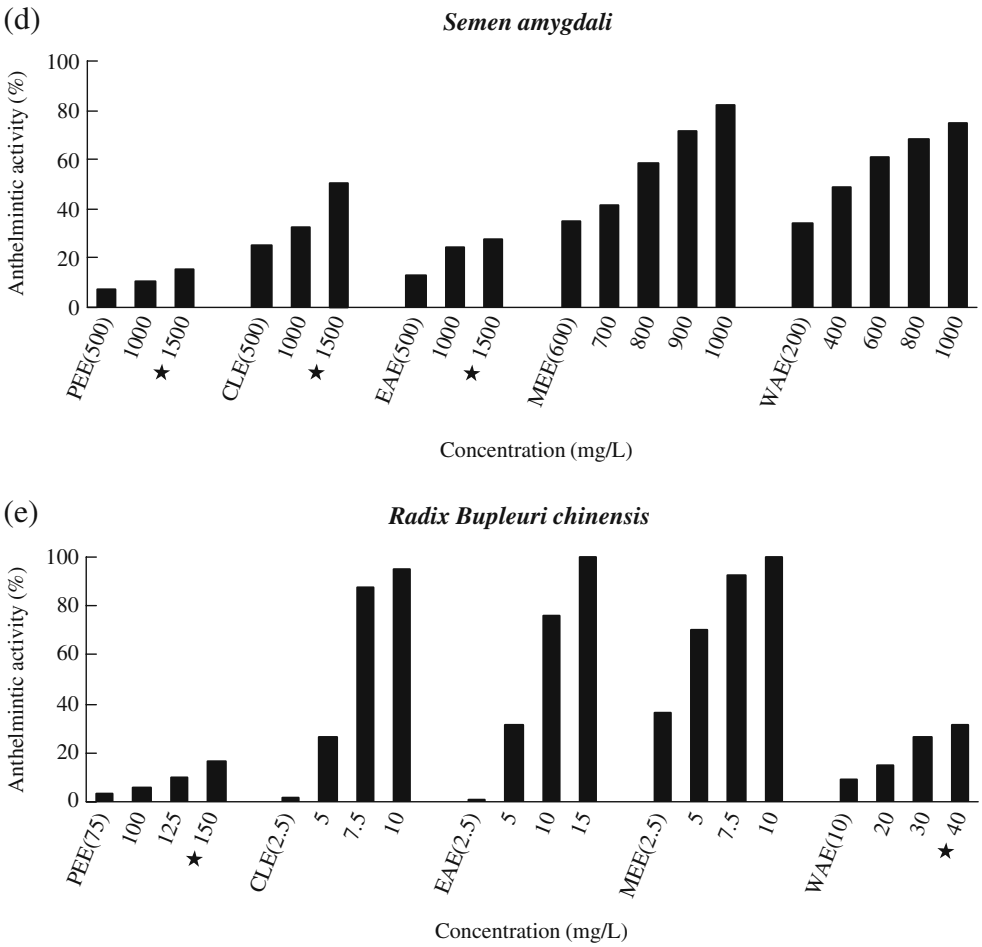


Fig. 1 Anthelmintic efficacy of different extracts of *Rhizoma cimicifugae*, *Radix peucedani*, *Semen momordicae*, *Semen amygdali*, and *Radix bupleuri chinensis* against *Dactylogyrus intermedius* after 48 h. *PEE* petroleum ether extract, *CLE* chloroform extract, *EAE* ethyl acetate extract, *MEE* methanol extract, *WAE* water extract, *star* indicates when fish mortality firstly occurred

Fig. 1 (continued)



were summarized in Table 3, which indicated that the three extracts were low toxic to goldfish. The 48 h LC_{50} of these crude extracts were 8.4-, 4.2- and 10.1-fold higher than the corresponding EC_{50}, with the values of 50.3, 31.4 and 35.2 mg/L, respectively.

Discussion

Infectious diseases caused by parasites are constraining the development and sustainability of the aquaculture sector through direct production losses and increased operating

Table 2 Anthelmintic efficacy of extracts from *Rhizoma cimicifugae*, *Radix peucedani*, *Semen momordicae*, *Semen amygdali*, and *Radix bupleuri chinensis* against *Dactylogyrus intermedius* after 48 h of exposure

Plants materials (vernacular names)	Solvents	EC_{50} (mg/L)	95% CL	EC_{90} (mg/L)	95% CL	χ^2
Rhizoma cimicifugae (Chinese: *Sheng Ma*)	Chloroform	267.8	114.8–373.5	852.5	709.0–1118.4	0.833
	Ethyl acetate	189.2	134.7–229.5	402.0	351.3–486.4	2.697
	Methanol	759.6	715.3–803.9	992.1	928.6–1094.8	3.632
Radix peucedani (Chinese: *Qian Hu*)	Chloroform	240.4	205.4–275.1	433.9	373.3–564.3	4.635
Semen momordicae (Chinese: *Mu Bie Zi*)	petroleum	724.8	681.2–790.7	950.8	860.6–1160.4	3.274
	Water	685.4	622.0–776.2	>1000.0	–	0.278
Semen amygdali (Chinese: *Bian Tao*)	Methanol	735.9	645.3–798.4	>1000.0	–	0.239
	Water	448.2	162.1–605.3	>1000.0	–	0.368
Radix bupleuri chinensis (Chinese: *Chai Hu*)	Chloroform	6.0	5.4–6.6	8.4	7.7–9.5	3.793
	Ethyl acetate	7.4	6.4–8.4	11.2	9.9–13.3	3.755
	Methanol	3.5	2.3–4.3	6.9	6.0–8.6	0.319

EC_{50} effective concentration 50%; *EC_{90}* effective concentration 90%; *95% CL* 95% confidence limit; χ^2 chi-square; (–) not calculated

Table 3 48 h acute toxicity of chloroform, ethyl acetate, and methanol extracts from *Radix bupleuri chinensis* against goldfish

Samples	LC_{50}	95% CL	LC_{90}	95% CL	χ^2
Chloroform extract	50.3	46.3–54.2	66.3	61.2–75.0	2.336
Ethyl acetate extract	31.4	29.4–33.6	39.9	37.1–44.9	1.960
Methanol extract	35.2	33.6–36.8	41.0	39.0–44.5	2.088

LC_{50} lethal concentration 50%; *LC_{90}* lethal concentration 90%; *95% CL* 95% confidence limit; χ^2 chi-square

costs, and indirectly, through restrictions on trade and impacts on biodiversity, consequently impede both economic and social development in many countries (Bondad-Reantaso et al. 2005). Among the parasites, the monogeneans, such as *D. intermedius*, are a problem because of their obvious pathogenicity and low susceptibility to chemicals. Numerous chemotherapeutic agents, including trichlorfon, formalin, toltrazuril, praziquantel, and mebendazole, have been widely used in aquaculture to treat the monogenean infestations (Goven et al. 1980; Schmahl et al. 1988; Treves-Brown 1999; Klinger and Floyd 2002). However, the frequent use of these chemical-based agents resulted in the raised drug-resistance and was often accompanied by serious drawbacks, including environmental contamination and even drug residues in fish products, which have led the use of these chemicals to be not recommended. Medicinal plants may be alternative sources of anti-monogenean agents, since they constitute a rich source of bioactive compounds that are biodegradable into non-toxic products and potentially suitable for use in control of parasites (Zahir et al. 2009). For this reason, it is necessary to test those plant extracts, which could contain substances of environmental potential and provide adequate efficacy against parasitic monogenean. Ekanem et al. (2004a) have found that the methanolic extracts of the seeds of *Piper guineense* (Piperaceae) were active against skin and gill monogenean parasites. The Indian almond leaf extract was also reported to exhibit a complete elimination of *Dactylogyrus* (Chansue 2007).

In the present study, results from the in vivo anthelmintic efficacy assay indicated that the crude methanolic extract of *R. Bupleuri chinensis* exhibited the most potent efficacy against *D. intermedius* with the least EC_{50} value of 3.5 mg/L after exposure for 48 h. In our previous work, the anthelmintic efficacies of different solvent extracts of *Radix angelicae pubescentis*, *Fructus bruceae*, *Caulis spatholobi*, *Semen aesculi*, and *Semen pharbitidis* against *D. intermedius* were also evaluated; and the results demonstrated that the methanolic and aqueous extracts from *S. aesculi* presented the best anthelmintic activity among the tested products, with the 48-h EC_{50} of 5.2 and 6.5 mg/L, respectively (Liu et al. 2010). These data indicated that the methanolic extract of *R. Bupleuri chinensis* is most effective in all these plant products tested, and *D. intermedius* is extremely sensitive to this extract, since it is 100% effective against the parasite when tested at lower dose (10 mg/L). With respect to the acute toxicity to the host, the results obtained ensure safety for the use of this methanol extract in controlling *D. intermedius* due to that the toxic dose is about ten times the effective one. In addition, a very important aspect to be taken into consideration is the higher availability of *R. Bupleuri chinensis*, which is produced in large commercial scale in China and other Asian countries. It enhances the possibility of the development and use of commercial products containing this material.

Radix bupleuri chinensis ("*Chai Hu*" in Chinese, meaning Chinese Thorowax Root), the dry roots of *Bupleurum chinense* DC (Umbelliferae), is an important Chinese herb in the treatment of influenza, fever, malaria, hepatitis, jaundice, nephritis, dizziness, bitter taste in the mouth, lung disease, cancer, and menstrual disorders in China, Japan, and other Asian countries (Zhao et al. 2009). Cheng et al. (2003) have reported that the acetone extract of *Bupleurum* sp. showed a dose-dependently antiproliferative effect on the proliferation of A549 human lung cancer cells. Anticomplementary activity produced by the hot-water extract of the roots of *Bupleurum smithii* have also been found (Xu et al. 2007). The major constituents, associated with the pharmacological activity of *R. bupleuri chinensis*, include triterpenoid glycosides of saikosaponin, essential oil, and polysaccharides (Tian et al. 2009). These saikosaponins are mainly found in the fat-soluble fractions and acknowledged to be the principal bioactive components, with saikosaponins a and d, as the dominant ones (Tian et al. 2009). Tundis et al. (2009) reported that saikosaponins a can exhibit strong cytotoxicity against cancer cell line COR-L23 with IC_{50} value of 0.6 μM. In the case of polysaccharides, Cheng et al. (2010) pointed out that at least part of the traditional beneficial effects of *Bupleurum* on inflammatory diseases could be ascribed to the immunomodulatory effects of *Bupleurum* polysaccharides on macrophages, based on the results obtained in a study that BALB/c mice were administered intragastrically with *Bupleurum* polysaccharides for 7 days. Although no attempts were made in this study to identify the com-

pound(s) responsible for the observed anthelmintic activity, some of the substances mentioned above are believed to contribute jointly or independently to the efficacy of the extracts from *R. bupleuri chinensis*. Additionally, the variation in activity of the extract types of this product might be due to the difference in the proportion of the active components responsible for the tested anthelmintic activity resulting from the difference in solubility in different solvents (Eguale et al. 2007).

In conclusion, the results obtained in this study showed that the methanolic extract from *R. bupleuri chinensis* exhibited the best elimination percentage of *D. intermedius* in goldfish (*C. auratus*), which afforded ground information for the potential use of this crude extract in controlling monogenean infections. Further bioassay-guided isolation and purification of compound(s) responsible for the observed anthelmintic efficacy are in progress.

Acknowledgments This work was supported by the National Natural Science Foundation of China (No. 31072242).

References

Bondad-Reantaso MG, Subasinghe RP, Arthur JR, Ogawa K, Chinabut S, Adlard R, Tan Z, Shariff M (2005) Disease and health management in Asian aquaculture. Vet Parasitol 132:249–272

Chansue N (2007) Effects of dried Indian almond leaf (*Terminalia catappa* L.) extract on monogenean parasites in goldfish (*Carassius auratus*). Vet Med Austria Wien Tierärztl Monatsschr 94:269–273

Cheng YL, Chang WL, Lee SC, Liu YG, Lin HC, Chen CJ, Yen CY, Yu DS, Lin SZ, Harn HJ (2003) Acetone extract of *Bupleurum scorzonerifolium* inhibits proliferation of A549 human lung cancer cells via inducing apoptosis and suppressing telomerase activity. Life Sci 73:2383–2394

Cheng XQ, Li H, Yue XL, Xie JY, Zhang YY, Di HY, Chen DF (2010) Macrophage immunomodulatory activity of the polysaccharides from the roots of *Bupleurum smithii* var. parvifolium. J Ethnopharmacol 130:363–368

Dove A, Ernst I (1998) Concurrent invaders—four exotic species of Monogenea now established on exotic freshwater fishes in Australia. Int J Parasitol 28:1755–1764

Eguale T, Tilahun G, Debella A, Feleke A, Makonnen E (2007) In vitro and in vivo anthelmintic activity of crude extracts of *Coriandrum sativum* against *Haemonchus contortus*. J Ethnopharmacol 110:428–433

Ekanem AP, Wang M, Simon JE, Obiekezie1 AI, Morah F (2004a) In vivo and in vitro activities of the seed extract of *Piper guineense* schum. and Thonn. against skin and gill monogenean parasites of goldfish (*Carassius auratus auratus*). Phytother Res 18:793–797

Ekanem AP, Obiekezie A, Kloas W, Knopf K (2004b) Effects of crude extracts of Mucuna pruriens (Fabaceae) and Carica papaya (Caricaceae) against the protozoan fish parasite Ichthyophthirius multifiliis. Parasitol Res 92:361–366

Finney DJ (1971) Probit analysis, 3rd edn. Cambridge University Press, Cambridge

Goven B, Gilbert J, Gratzek J (1980) Apparent drug resistance to the organophosphate dimethyl (2, 2, 2-trichloro-1-hydroxyethyl) phosphonate by monogenetic trematodes. J Wildl Dis 16 (3):343–346

Klinger R, Floyd RF (2002) Introduction to freshwater fish parasites. Document CIR716. Institute of Food and Agricultural Science. University of Florida, Florida

Liu YT, Wang F, Wang GX, Han J, Wang Y, Wang YH (2010) In vivo anthelmintic activity of crude extracts of *Radix angelicae pubescentis*, *Fructus bruceae*, *Caulis spatholobi*, *Semen aesculi*, and *Semen pharbitidis* against *Dactylogyrus intermedius* (Monogenea) in goldfish (*Carassius auratus*). Parasitol Res 106:1233–1239

Reed PA, Francis-Floyd R, Klinger RC (2009) FA28/FA033. Monogenean parasites of fish. EDIS—Electronic Data Information Source—UF/IFAS Extension. University of Florida. http://edis.ifas.ufl.edu/FA033.17 May 2009

Sapkota A, Sapkota AR, Kucharski M, Burke J, McKenzie S, Walker P, Lawrence R (2008) Aquaculture practices and potential human health risks: current knowledge and future priorities. Environ Int 34:1215–1226

Schmahl G, Mehlhorn H (1985) Treatment of fish parasites. 1. Praziquantel effective against Monogenea (*Dactylogyrus vastator*, *Dactylogyrus extensus*, *Diplozoon paradoxum*). Z Parasitenk 71:727–737

Schmahl G, Mehlhorn H, Haberkorn A (1988) Sym. Triazinone (toltrazuril) effective against fish-parasitizing Monogenea. Parasitol Res 75(1):67–68

Thoney DA, Hargis WJ (1991) Monogenea (Platyhelminthes) as hazards for fish in confinement. Annu Rev Fish Dis 1:133–153

Tian RT, Xie PS, Liu HP (2009) Evaluation of traditional Chinese herbal medicine: Chaihu (*Bupleuri Radix*) by both high-performance liquid chromatographic and high-performance thin-layer chromatographic fingerprint and chemometric analysis. J Chromatogr A 1216:2150–2155

Tóro RM, Gessner AAF, FurtadoNAJC CPS, Albuquerque SD, Bastos JK (2003) Activity of the *Pinus elliottii* resin compounds against *Lernaea cyprinacea* in vitro. Vet Parasitol 118:143–149

Treves-Brown KM (1999) Availability of medicines for fish. Fish Vet J 4:40–55

Tundis R, Bonesi M, Deguin B, Loizzo MR, Menichini F, Conforti F, Tillequin F, Menichini F (2009) Cytotoxic activity and inhibitory effect on nitric oxide production of triterpene saponins from the roots of *Physospermum verticillatum* (Waldst & Kit) (Apiaceae). Bioorgan Med Chem 17:4542–4547

Wang GX, Zhou Z, Cheng C, Yao JY, Yang ZW (2008) Osthol and isopimpinellin from *Fructus cnidii* for the control of *Dactylogyrus intermedius* in *Carassius auratus*. Vet Parasitol 158:144–151

Wang GX, Han J, Feng TT, Li FY, Zhu B (2009) Bioassay-guided isolation and identification of active compounds from *Fructus Arctii* against *Dactylogyrus intermedius* (Monogenea) in goldfish (*Carassius auratus*). Parasitol Res 106:247–255

Wang GX, Jiang DX, Li J, Han J, Liu YT, Liu XL (2010a) Anthelmintic activity of steroidal saponins from *Dioscorea zingiberensis* C. H. Wright against *Dactylogyrus intermedius* (Monogenea) in goldfish (*Carassius auratus*). Parasitol Res 107:1365–1371

Wang GX, Han J, Zhao LW, Jiang DX, Liu YT, Liu XL (2010b) Anthelmintic activity of steroidal saponins from *Paris polyphylla*. Phytomedicine 17:1102–1105

Woo PTK, David W, Bruno LH, Susan L (2002) Diseases and disorders of finfish in cage culture. CABI, Malaysia

Xu H, Zhang Y, Zhang J, Chen D (2007) Isolation and characterization of an anti-complementary polysaccharide D3-S1 from the roots of *Bupleurum smithii*. Int Immunopharmacol 7:175–182

Zahir AA, Rahuman AA, Kamaraj C, Bagavan A, Elango G, Sangaran A, Kumar BS (2009) Laboratory determination of efficacy of indigenous plant extracts for parasites control. Parasitol Res 105:453–461

Zhao R, Liu S, Mao S, Wang Y (2009) Study on liver targeting effect of vinegar-baked *Radix Bupleuri* on resveratrol in mice. J Ethnopharmacol 126:415–420