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Research Article

Screening of Chinese Medicinal Herbs for Anthelmintic Efficacy Against *Gyrodactylus kobayashii* (Monogenea) in Goldfish *(Carassius auratus)*

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Abstract

Monogenean infection can cause high mortality and significant financial losses in commercially farmed fish. Existing chemical drugs for the treatment of such infections often have serious drawbacks. In order to find alternative agents of chemical drugs, fourteen medicinal plants were tested for their in vivo anthelmintic activity against *Gyrodactylus kobayashii* in goldfish *(Carassius auratus)*. Ethanol extracts of *Evodia rutaecarpa, Cnidium monnieri* and *Sophora flavescens* had 100% anthelmintic efficacy at low concentrations (100, 100 and 300 mg/L, respectively), after 48 h of exposure. The ethyl acetate extract of *C. monnieri* was the most effective, with an EC_{50} value of 11.0 mg/L, after 48 h of exposure, showing 100% anthelmintic efficacy against *G. kobayashii* at 50.0 mg/L. Higher anthelmintic activity was also observed for remaining extracts of *C. monnieri* and *E. rutaecarpa* except for the water extracts of the two plants. The ethyl acetate extract of *C. monnieri* had the highest therapeutic index (TI, LC_{50}/EC_{50}) value of 31.8, and the lowest EC_{50}, which indicates that this extract was the safest to goldfish among all extracts. For the remaining extracts of *C. monnieri* and *E. rutaecarpa,* the 48-h LC_{50} values were about 10-fold higher than the corresponding EC_{50} values. This result indicates these extracts have low toxicity in goldfish. The ethyl acetate extract of *C. monnieri* was the most effective and the safest among the tested extracts. Therefore, the two plants are expected to be alternative agents to control monogenean infection.

Keywords: *Disease control, Gyrodactylus kobayashii, Chinese Medicinal Herb, Goldfish, Anthelmintic efficacy*

Japon Balıklarında *(Carassius auratus) Gyrodactylus kobayashii*'ye (Monogenea) Karşı Çin Şifalı Otlarının Anthelmintik Etkinliklerinin İncelenmesi

Öz

Monogenean enfeksiyonları, ticari olarak yetiştirilen balıklarda yüksek mortalite ve önemli finansal kayıplara neden olabilir. Bu tür enfeksiyonların tedavisi için mevcut kimyasal ilaçların genellikle ciddi dezavantajları vardır. Kimyasal ilaçların alternatif ajanlarını belirleyebilmek için on dört şifalı bitki, akvaryum balığındaki *(Carassius auratus)* Gyrodactylus kobayashii'ye karşı in vivo antelmintik aktiviteleri açısından test edildi. *Evodia rutaecarpa, Cnidium monnieri* ve *Sophora flavescens*'in etanol özleri, 48 saat maruziyetten sonra düşük konsantrasyonlarda (sırasıyla 100, 100 ve 300 mg/L) %100 antelmintik etkinliğe sahipti. *C. monnieri*'nin etil asetat özütü, 48 saat maruziyetten sonra EC_{50} değeri 11.0 mg/L ile en etkili olanıydı ve 50.0 mg/L'de *G. kobayashii*'ye karşı %100 antelmintik etkinlik gösterdi. *C. monnieri* ve *E. rutaecarpa*'nın su özütleri dışındaki özütleri için de daha yüksek antelmintik aktivite gözlemlendi. *C. monnieri*'nin etil asetat ekstresi, en yüksek terapötik indeks (TI, LC_{50}/EC_{50}) değeri 31.8 ve en düşük EC_{50}'ye sahipti, ve bu bulgu bu ekstraktın tüm ekstraktlar arasında akvaryum balığı için en güvenli olduğunu gösterdi. *C. monnieri* ve *E. rutaecarpa*'nın geri kalan ekstraktları için 48 saatlik LC_{50} değerleri, ilgili EC_{50} değerlerinden yaklaşık 10 kat daha yüksekti. Bu sonuç, bu ekstraktların Japon balıklarında düşük toksisiteye sahip olduğunu gösterdi. *C. monnieri*'nin etil asetat özütü, test edilen özütler arasında en etkili ve en güvenli olanıydı. Bu nedenle, iki bitki Monogenean enfeksiyonlarının kontrolünde alternatif ajanlar olabilir.

Anahtar sözcükler: *Hastalık kontrolü, Gyrodactylus kobayashii, Çin şifalı bitki, Japon balığı, Antelmintik etkinlik*

INTRODUCTION

Ornamental fish trade is one of the most rapidly developing areas of the aquaculture industry, and the value of international trade exports of ornamental fish has grown by an average of about 14% per year since 1985 [1,2]. The

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rapid development of the ornamental fish industry has been overwhelmed by the occurrence of large-scale parasitic diseases. Goldfish *(Carassius auratus)* is one of most popular ornamental fish worldwide, due to its easy maintenance and its attractive coloration. The most common ecto-parasites infecting goldfish are gyrodactylids [3]. Viviparous gyrodactylids have a direct life-cycle. They can directly spread among hosts by contact, and undergo continuous transmission throughout their life-cycle [4,5]. Serious infection can cause evident clinical symptoms [3]. Monogeneans can damage the fish epidermis, which results in secondary infections by other pathogenic microorganisms [6,7].

It is difficult to control monogeneans. Many chemical drugs have been used against these parasites, but bring some drawbacks (e.g., low efficacy, toxicity to host and environmental and human health problems) [8,9]. Additionally, the long-term use of chemical drugs can lead to drug resistance in parasites [10]. Recently, increasing attention has been paid to the use of traditional plant-based medicines to control diseases in aquaculture [11,12]. Zhou et al.[13] showed that herbal medicines were able to control *Gyrodactylus kobayashii (G. kobayashii)* infection in goldfish. Huang et al.[14] screened plant extracts with anthelmintic activity against *Dactylogyrus intermedius* (Monogenea) in goldfish.

In order to find safe and efficient alternatives to treat monogenean infection in aquaculture, we evaluated the anthelmintic efficacy of ethanol extracts of 14 plants against *G. kobayashii* in goldfish, using *in vivo* anthelmintic efficacy assays.

MATERIAL and METHODS

Establishment of a Goldfish-G. kobayashii Model and Animal Ethics

Goldfish weighing 3.7±0.7 g were selected from a fish farm in Anyang city, Henan province, China. All experiments complied with institutional animal care guidelines and were approved by the Animal Care Committee of Anyang Institute of Technology and Academician Workstation of Animal Disease Control and Nutrition Immunity in Henan Province (License no. SCXK(AWADCNI)2018-0001). These goldfish were fed and treated to remove all ectoparasites, as described previously [13]. A goldfish experimental infection model using *G. kobayashii* was performed according to a method described previously [15]. Uninfected fish were anesthetized with 0.02% MS-222. In order to allow parasite transfer between hosts, the caudal fins of uninfected fish were placed in contact with the caudal fins of heavily-infected fish reared in our laboratory. Experimentally-infected fish were then placed in a 1 L container. The success of the infection was determined by daily examination of the fins using a stereomicroscope. Ten days after infection, two parasites were collected from the infected fish for morphological and molecular identification according to Li et al.[16] in order to verify that the infective agent was *G. kobayashii*. Uninfected goldfish were introduced periodically into an 80 L aquarium containing infected goldfish, in order to increase the number of infected fish for the experiments.

Preparation of Plant Materials

The fourteen Chinese medicinal herbs listed in *Table 1* were purchased from a drug store and prepared according to a method described previously [13]. The herbal medicines were washed thoroughly, air-dried and oven-dried at 45°C for 48 h. In order to ensure the complete removal of water, dry plant materials were powdered, strained using a filter net of 30-40 mesh (450-600 μm), and freeze-dried at -54°C. The powder of all the medicinal plants was extracted with ethanol. The powder of *E. rutaecarpa*, *C. monnieri* and *S. flavescens* was extracted for 48 h with water, methanol, petroleum ether, or ethyl acetate; the extraction process was repeated three times. To obtain solidified crude extracts, the extract solutions were filtered, and then concentrated at reduced pressure in a vacuum rotary evaporator, until all solvents had evaporated. Solid extracts then were dissolved in dimethyl sulfoxide (DMSO) at 0.5 mg/L as stock solutions for anthelmintic efficacy assays.

Screening Experiment

The dry powder (50.0 g) of herbal medicines was extracted three times with 500.0 mL ethanol for 48 h. Ethanol filtrates were evaporated at reduced pressure in a vacuum rotary evaporator in order to obtain solidified crude extracts [14]. The solidified crude extract of each plant was dissolved in DMSO as a stock solution at 0.5 g/mL; these stock solutions were then screened for anthelmintic efficacy.

In addition, three kinds of herbal medicines *(E. rutaecarpa*, *C. monnieri* and *S. flavescens)* with higher antiparasitic activities *(Table 1)* were fractionated with different solvents (water, methanol, petroleum ether, and ethyl acetate), and then tested in anthelmintic efficacy assays. Each solid extract was dissolved in DMSO to obtain the stock solution at the concentration of 0.5 g/mL, which was used in anthelmintic efficacy assays.

In vivo Anthelmintic Efficacy Assay

The anthelmintic assay against *G. kobayashii* in goldfish was performed according to a previous method [13]. Two goldfish infected with *G. kobayashii* were placed in 20×12×10 cm tanks containing 0.5 L of the test solutions at 23±1°C for 48 h. Two control groups without extract, or with the highest percentage of DMSO, were set at the same experimental conditions. All treatments and control groups were performed using five replicates. Before manipulation, goldfish were anesthetized with 0.02% MS-222, and the parasites in the caudal fin were counted under a stereomicroscope.

Table 1. *The tested medicinal plants and the anthelmintic efficacy (AE) against Gyrodactylus kobayashii in goldfish of the ethanol extracts*

Species	Plant Part Used	Mean Intensity (±SD)	The Best AE (%)	The Concentrations of the Best AE (mg/L)	The Concentrations of Fish Died (mg/L)
Evodia rutaecarpa (Juss.) Benth.	Fruit	102.6±45.2	100	100	500
Cnidium monnieri (L.) Cuss.	Fruit	87.6±35.4	100	100	200
Sophora flavescens Ait.	Root	95.9±50.4	100	300	500
Areca catechu L.	Seed	101.8±39.6	100	150	150
Citrus reticulate Blanco	Bark	96.9±51.5	100	400	800
Mentha haplocalyx Briq.	Leaf	117.8±40.1	100	400	1000
Punica granatum L.	Bark	105.8±42.4	100	600	800
Agrimonia pilosa Ledeb.	Aerialparts	109.1±37.1	100	600	600
Omphalia lapidescens Schroet.	Sclerotium	112.3±43.4	100	800	800
Quisqualis indica L.	Fruit	82.7±30.4	94.7	800	1000
Dryopteris crassirhizoma Nakai	Root, stem and leaf	101.7±25.7	89.0	200	200
Pharbitis nil (L.) Choisy	Seed	95.9±28.5	54.4	200	200
Stemona sessilifolia (Miq.) Miq.	Root	91.1±24.0	43.3	700	700
Cynanchum paniculatum (Bge) Kitaga	Rhizome	98.1±39.1	9.9	300	300

The anthelmintic efficacy of each extract was calculated according to the following formula: $E = (L–L_t)/L\times100\%$ for $L>L_t$, $E = 0$ for $L\leq L_t$. E is anthelmintic efficacy, L is the number of *G. kobayashii* on the caudal fin before treatment, and L_t is the number of surviving parasites after the treatment. Mean anthelmintic efficacy was calculated from five replicates per treatment.

Acute Toxicity Tests

The extract with the strongest anthelmintic efficacy in *in vivo* anthelmintic efficacy assay, was tested for safety to goldfish. Acute toxicity tests were conducted in 26.5×16.5×12.5 cm tanks, containing ten healthy goldfish, and 2 L of aerated tap water at 23±1°C. Fish in tanks without plant extracts constituted the control group. Three replicates were set for the group of control fish and for the groups of fish exposed to plant's extracts. Fish mortality in each tank was recorded after 48 h. Fish were not fed during the experiment, and dead fish were continuously removed in order to avoid deterioration of water quality.

Statistical Analyses

The homogeneity of replication samples was evaluated through the Mann-Whitney U test. At the 95% confidence level, median lethal concentration (LC_{50}, LC_{90}) and median effective concentration (EC_{50}, EC_{90}) were determined using the probit analysis. The therapeutic index (TI) was calculated as LC_{50}/EC_{50}. Each statistical analysis was performed using SPSS 19.0. A value of $P<0.05$ was considered significant and $P<0.01$ was considered highly significant.

RESULTS

Anthelmintic Efficacy of Extracts Against G. kobayashii In vivo

As shown in *Table 1*, of the 14 medicinal plants selected, only the ethanol extracts of *E. rutaecarpa*, *C. monnieri* and *S. flavescens* had 100% anthelmintic efficacy at 100.0, 100.0 and 300.0 mg/L, respectively, after 48 h of exposure. Additionally, the extracts of *Citrus reticulate*, *Mentha haplocalyx*, *Punica granatum*, *Agrimonia pilosa* and *Omphalia lapidescens* showed good anthelmintic efficacy, but only at high concentrations *(Table 1)*. There was either very weak anthelmintic activity, or highly toxicity to goldfish, in the remaining ethanol extracts of the herbal medicines tested. In the control groups, the number of *G. kobayashii* increased on the caudal fin (from 98.2±25, 7 to 160.5±42.7 in the group with no extract, and from 113.1±21.5 to 150.5±31.3 in the DMSO group).

For the different extracts of *E. rutaecarpa*, *C. monnieri* and *S. flavescens*, their anthelmintic efficacy is showed in *Fig. 1, 2, 3,* and the corresponding EC_{50} and EC_{90} values are showed in *Table 2*. The ethyl acetate extract of *C. monnieri* exhibited 100% anthelmintic efficacy against *G. kobayashii* at 50.0 mg/L, and was the most effective against *G. kobayashii*, with an EC_{50} value of 11.1 mg/L, and an EC_{90} value of 28.3 mg/L, after 48 h of exposure. In addition, the anthelmintic activity against *G. kobayashii* was also determined for the methanol and petroleum ether extracts of *C. monnieri*, with EC_{50} values of 23.6 and 44.6 mg/L, and EC_{90} values of 51.0 and 84.0 mg/L, respectively. In all the extracts of *C. monnieri*, the water extract had the weakest anthelmintic efficacy of 42.7% at 1 000.0 mg/L.

The ethyl acetate extract of *E. rutaecarpa* had good anthelmintic efficacy, with EC_{50} and EC_{90} values of 24.0 and 50.3 mg/L, respectively, after a 48-h exposure. The petroleum ether and methanol extracts of *E. rutaecarpa* showed 100% anthelmintic efficacy at 150.0 and 120.0 mg/L, with EC_{50} and EC_{90} values of 71.9 and 108.3 mg/L (petroleum ether), and 40.9 and 91.2 mg/L (methanol), respectively. The water extract of *E. rutaecarpa* had the weakest anthelmintic efficacy of 25.6% at 800.0 mg/L. All

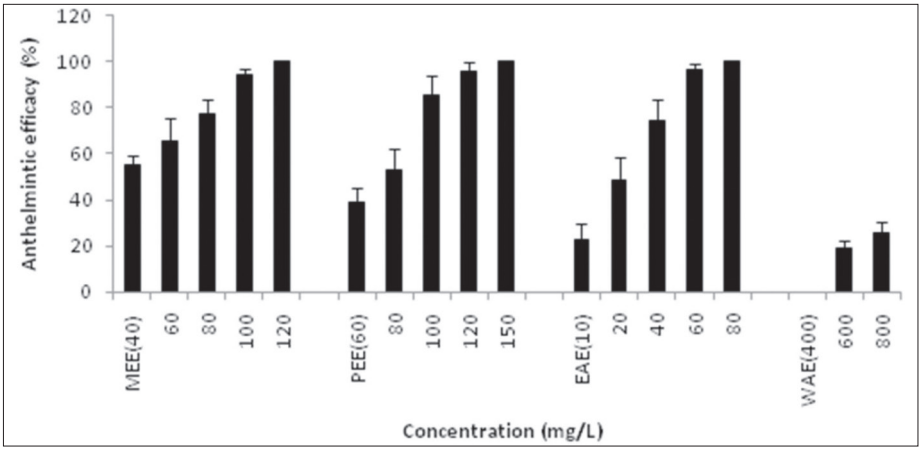
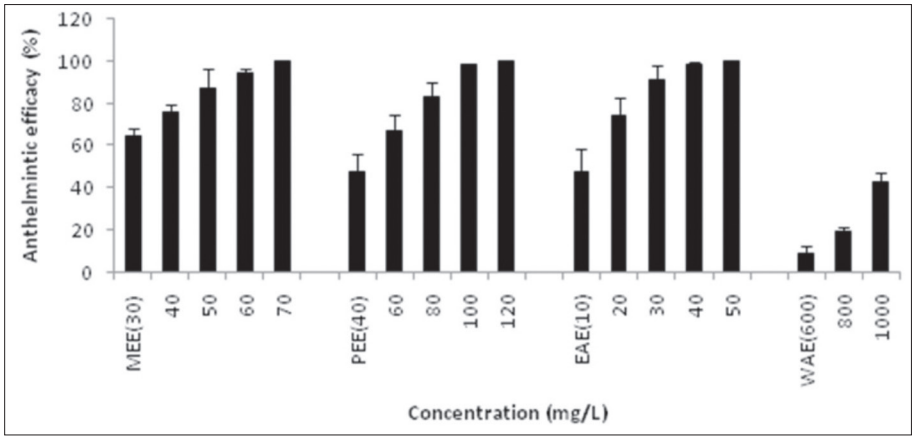


Fig 1. Anthelmintic activity of the different extracts of *Evodia rutaecarpa* against *Gyrodactylus kobayashii* after the exposure for 48 h. MEE, methanol extract; EAE, ethyl acetate extract; PEE, petroleum ether extract; WAE, water extract

Fig 2. Anthelmintic activity of the different extracts of *Cnidium monnieri* against *Gyrodactylus kobayashii* after the exposure for 48 h. MEE, methanol extract; EAE, ethyl acetate extract; PEE, petroleum ether extract; WAE, water extract



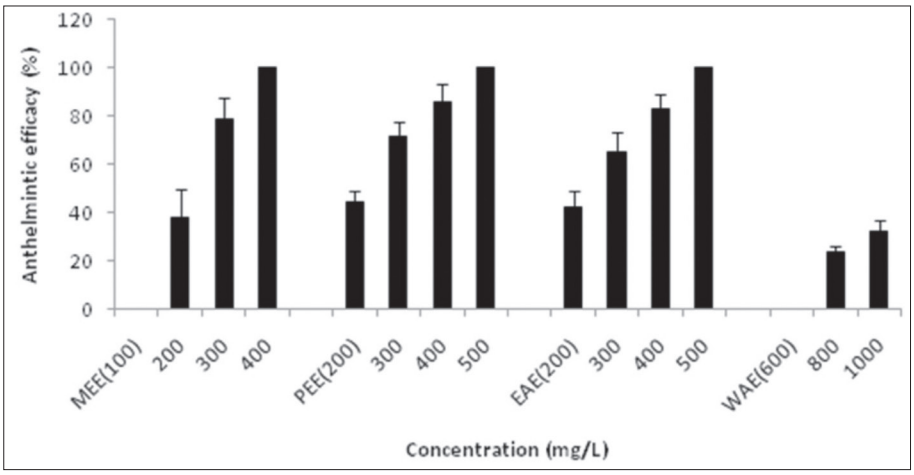


Fig 3. Anthelmintic activity of the different extracts of *Sophora flavescens* against *Gyrodactylus kobayashii* after the exposure for 48 h. MEE, methanol extract; EAE, ethyl acetate extract; PEE, petroleum ether extract; WAE, water extract

the extracts of *S. flavescens* showed effective anthelmintic efficacy against *G. kobayashii* after 48 h of exposure, but at high concentrations, with EC_{50} values of 234.6 mg/L (methanol), 224.4 mg/L (petroleum ether) and 238.6 mg/L (ethyl acetate).

Acute Toxicity of the Tested Extracts in Goldfish

The results of the acute toxicity assay of the extracts with higher anthelmintic efficacy at the low concentrations (methanol, petroleum ether, and ethyl acetate extracts of *E. rutaecarpa* and *C. monnieri*) are summarized in *Table 3*. After 48 h of exposure, the LC_{50} values of the extracts of *E. rutaecarpa* were 759.3 (methanol), 771.9 (petroleum ether) and 235.9 (ethyl acetate) mg/L, which is 18.6, 10.7 and 9.9 times higher than the corresponding EC_{50}, respectively. The methanol, petroleum ether, and ethyl acetate extracts of *C. monnieri* had LC_{50} values of 214.1, 405.3 and 350.0 mg/L, which is 9.1-, 9.1- and 31.8 times higher than the corresponding EC_{50}, respectively. The water extracts of *E. rutaecarpa* and *C. monnieri* exhibited very weak toxicity to goldfish, and killed only two and

Table 2. *Anthelmintic efficacy (EC_{50} and EC_{90}) of different extracts from Evodia rutaecarpa, Cnidium monnieri and Sophora flavescens against Gyrodactylus kobayashii after 48 h of exposure*

Plants	Extraction Solvent	EC_{50} (mg/L)	95% CI	EC_{90} (mg/L)	95% CI	P value
Evodia rutaecarpa	Methanol	40.9	0-56.0	91.2	76.3-131.1	0.06
	Petroleum ether	71.9	66.7-76.2	108.3	102.8-115.7	0.23
	Ethyl acetate	24.0	20.8-26.9	50.3	46.0-56.0	0.38
Cnidium monnieri	Methanol	23.6	15.8-28.4	51.0	47.6-55.8	0.46
	Petroleum ether	44.6	37.8-49.8	84.0	78.5-91.4	0.25
	Ethyl acetate	11.1	7.7-13.5	28.3	25.82-31.69	0.94
Sophora flavescens	Methanol	234.6	161.5-303.0	319.8	266.0-543.6	0.07
	Petroleum ether	224.4	0-292.8	400.0	328.1-714.7	0.11
	Ethyl acetate	238.6	0-315.3	416.4	335.9-914.6	0.06

EC_{50}, effective concentration with 50% anthelmintic efficacy; EC_{90}, effective concentration with 90% anthelmintic efficacy; 95% CI, 95% confidence interval

Table 3. *Acute toxicity for goldfish exposed to different extracts from Evodia rutaecarpa and Cnidium monnieri after 48 h of exposure*

Plants	Extraction Solvent	LC_{50} (mg/L)	95% CI	LC_{90} (mg/L)	95% CI	TI (LC_{50}/EC_{50})	P value
Evodia rutaecarpa	Methanol	759.3	713.5-806.6	854.7	807.3-975.2	18.6	0.45
	Petroleum ether	771.9	719.6-826.5	892.8	835.5-1039.1	10.7	0.32
	Ethyl acetate	235.9	172.6-298.8	385.9	317.2-579.7	9.9	0.70
	Water	>1000.0	-	-	-	-	-
Cnidium monnieri	Methanol	214.1	186.8-240.2	274.6	246.7-344.5	9.1	0.70
	Petroleum ether	405.3	332.0-462.8	552.9	488.5-735.8	9.1	0.30
	Ethyl acetate	350.0	301.5-398.5	453.6	403.7-579.8	31.8	0.59
	Water	>1000.0	-	-	-	-	-

LC_{50}, 50% lethal concentration; LC_{90}, 90% lethal concentration; 95% CI, 95% confidence interval; TI, therapeutic index; –, not calculated

one goldfish, respectively, at the concentration of 1000.0 mg/L.

DISCUSSION

Diseases caused by the common ectoparasites, *Gyrodactylus*, can result in high mortality and huge financial losses in commercially farmed fish [6,17]. Additionally, prolonged and frequent use of common chemicals such as formaldehyde, rotenone, and praziquantel results in increasing drug resistance and adverse effects on the environment [8,10]. Therefore, there is an urgent need for effective strategies to control *Gyrodactylus* infections. In our study, the ethyl acetate extract of *C. monnieri* was the most efficient, having the lowest EC_{50} and EC_{90} values (11.0 and 28.3 mg/L, respectively) after 48 h of exposure, and it had 100% anthelmintic efficacy against *G. kobayashii* at 50.0 mg/L. The dried fruit of *C. monnieri* (L.) Cuss., called "Shechuangzi" in Chinese, is a commonly used traditional Chinese medicine with an impressive range of health benefits [18]. Previous research has found that the ethanol extract had better anthelmintic efficacy against *Dactylogyrus intermedius (D. intermedius*) in goldfish, and had the minimal effective concentration compared to the chloroform, petroleum ether, water, and acetone extracts of *C. monnieri*. This extract had 100% anthelmintic efficacy at 70.0 mg/L after 48 h of exposure. The active compounds of the ethanol extract against *D. intermedius* were identified as osthol and isopimpinellin [19,20]. In the present study, the ethyl acetate extract of *C. monnieri* was the most effective against *G. kobayashii* in goldfish at the minimal concentration, showing 100% anthelmintic efficacy at 50.0 mg/L, which was far lower than the concentration of ethanol extract with 100% anthelmintic efficacy (100.0 mg/L). Differences in anthelmintic efficacy of the same extracts may be due to the differences in the evaluation methods. In previous research, anthelmintic efficacy was calculated by comparison with the control group. In our paper, the number of *G. kobayashii* was recorded before and after exposure, and anthelmintic efficacy was calculated based on the changes in intensity of *Gyrodactylus* in goldfish. Besides, the difference in the main parasitic sites may be another cause. The main parasitic site of *Dactylogyrus* sp. is the gill, which may provide partial protection for this parasite by reducing the exposure to extracts. Nevertheless, *Gyrodactylus* sp. is mainly parasitic on the fins or the skin, which directly exposes this parasite to the extracts.

"Wuzhuyu", derived from the dried fruit of *E. rutaecarpa* (Juss.) Benth., is well described in the Chinese medical matter

and the Chinese pharmacopoeia, and it is recommended for the treatment of dizziness, headache, emesis, diarrhea and other symptoms [21,22]. Pharmacological studies indicate that the bioactive constituents of *E. rutaecarpa* have many properties, including anti-inflammatory, antihypertensive, antinociceptive, antimicrobial and anthelmintic activities [23,24]. For example, Perrett and Whitfield [25] reported that atanine (3-Dimethylallyl-4-methoxy-2-quinolone), an alkaloid isolated from the hexane extract of *E. rutaecarpa* caused 100% immobility of the cercarial and miracidial larvae of *Schistosoma mansoni* at 100.0 mg/L, after 7 min of exposure. Liu et al.[26] have shown that ethanol extracts of *E. rutaecarpa* are effective at killing intestinal nematode parasites of pigs and leeches *in vitro*. Also, the n-hexane extract of *E. rutaecarpa* has been demonstrated to have strong antifeedant activity against the grain storage insects *Sitophilus zeamais* and *Tribolium castaneum* [26]. Moreover, two alkaloids isolated from the methanol extract of *E. rutaecarpa*, evodiamine and rutaecarpine, have shown insecticidal activity against fruit flies (*Drosophila melanogaster*), with LC_{50} values of 3.9 and 3.6 mg per adult, respectively [27]. In the present study, the ethyl acetate extract of *E. rutaecarpa* showed good anthelmintic efficacy, with EC_{50} and EC_{90} values of 24.0 and 50.3 mg/L, after 48 h of exposure. Although the active compounds against *G. kobayashii* that are contained in the ethyl acetate extract have not been identified, some of the compounds described above are thought to jointly or independently control *G. kobayashii* infection.

The therapeutic index (TI), aparameter for the quantitative relationship between efficacy and safety, is used to evaluate the potential application of the extracts in aquaculture [28]. TI is calculated as the radio of LC_{50} to EC_{50}, and higher TI values reflect safer effects. In our study, we found that the ethyl acetate extract of *C. monnieri* had the highest TI value of 31.8, and the lowest EC_{50}, which indicated this extract was safest to goldfish among all extracts and had the potential to be used in aquaculture. Additionally, the methanol extract had a higher TI value of 18.6, but the EC_{50} was also higher compared with the ethyl acetate extract for *E. rutaecarpa*. The 48-h LC_{50} values of the remaining extracts of *C. monnieri* and *E. rutaecarpa* were about 10-fold higher than the corresponding EC_{50} values, which reflects low toxicity to goldfish. The bioactive ingredients that play a major role in anthelmintic activity are necessary for further bioassay-guided isolation and identification. In addition, field trials need to be performed before the use of extracts from *C. monnieri* and *E. rutaecarpa* in aquaculture.

Among the 14 Chinese medicinal herbs screened, the extracts of *C. monnieri* and *E. rutaecarpa* had the highest anthelmintic efficacy against *G. kobayashii* in goldfish. The ethyl acetate extract of *C. monnieri* was the most effective and the safest of all the extracts tested. *C. monnieri* and *E. rutaecarpa* may become novel therapeutic agents against *G. kobayashii* infection.

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Competing Interests

The authors declare they have no competing interests.

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