Parasitol Res (2012) 110:1085–1090
DOI 10.1007/s00436-011-2592-0

ORIGINAL PAPER

In vivo anthelmintic activity of *Dryopteris crassirhizoma*, *Kochia scoparia*, and *Polygala tenuifolia* against *Dactylogyrus intermedius* (Monogenea) in goldfish (*Carassius auratus*)

Cheng Lu · Hong-Yu Zhang · Jie Ji · Gao-Xue Wang

Received: 12 July 2011 / Accepted: 1 August 2011 / Published online: 14 August 2011

Abstract In order to find natural agents against *Dactylogyrus intermedius* in goldfish, petroleum ether, chloroform, ethyl acetate, acetone, and methanol extracts of three medicinal plants (*Dryopteris crassirhizoma*, *Kochia scoparia*, and *Polygala tenuifolia*) were screened for antiparasitic properties using in vivo anthelmintic efficacy assay. Among these extracts investigated, methanolic extract of *D. crassirhizoma* was observed the most effective with EC_{50} value of 22.97 mg L^{-1} after 48 h of exposure, which exhibited a 100% efficacy against *D. intermedius* at 60.00 mg L^{-1}, followed by the methanolic extracts of *K. scoparia* and *P. tenuifolia* with EC_{50} values of 31.28 and 154.79 mg L^{-1}, showing 100% efficacy against *D. intermedius* at 60.00 and 500.00 mg L^{-1}, respectively. In addition, acute toxicity assay indicated that 48-h LC_{50} values of methanolic extracts of *D. crassirhizoma*, *K. scoparia*, and *P. tenuifolia* were 4.10-, 2.27-, and 5.00-fold higher than the corresponding EC_{50}. The obtained results demonstrated that methanolic extracts of *D. crassirhizoma*, *K. scoparia*, and *P. tenuifolia* have the potential for the development of novel therapy for the control of *D. intermedius* in aquaculture.

Introduction

As an important economic activity, fish aquaculture has received increasing concern nowadays. However, with the expanding of aquaculture industries, the incidence of the event that the fish infected by the gill monogenean parasite, *D. intermedius*, is emerging (Topić et al. 2001; İsmail and Selda 2007). The fish heavily infected with *D. intermedius* would show many serious symptoms, such as pale gills, excessive mucous secretions, and increasing respiration rate (Reed et al. 2009). Furthermore, it may be more easily subjected to mixed infections with other parasites, bacterial, fungal, and viral, causing 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).

Various parasiticides, such as formalin, praziquantel, toltrazuril, and mebendazole (Marshall 1999; Schmahl and Mehlhorn 1985; Schmahl et al. 1988; Treves-Brown 1999), have been successfully used for the treatment against *D. intermedius* at varying concentration. However, due to the frequent use of these drugs, many serious drawbacks became visible including the raised drug resistance, environmental contamination, toxicity to the host, and even contamination of fish products with drug residues (Goven et al. 1980; Klinger and Floyd 2002). Thus, the alternative means of control are in urgent need.

Medicinal plants have long been used in traditional medicine in China. Currently, use of herbs for the control of parasitic infections in animals has become popular. Bagavan et al. (2010) found that the ethyl acetate and methanol extracts of *Leucas aspera* (leaf), acetone and methanol extracts of *Phyllanthus acidus* (seed), and acetone extract of *Terminalia chebula* (seed) had good antiplasmodial activity (IC_{50}=7.81, 22.76, 9.37, 14.65, 12.68, and 4.76 μg mL^{-1}). Our previous research had found crude extracts of several traditional medicinal plants can effectively control the *D. intermedius* infection in goldfish (*Carassius auratus*), such as *Arctium lappa* L., *Dioscorea zingiberensis* C. H. Wright, and *Paris polyphylla* (Wang et al. 2009; Wang et al. 2010a, b).

C. Lu · J. Ji · G.-X. Wang (✉)
Northwest A&F University,
Xinong Road 22nd, Yangling,
Xianyang, Shaanxi 712100, China
e-mail: wanggaoxue@126.com

H.-Y. Zhang
Chinese Academy of Fishery Sciences,
Yongding Road 150th,
Beijing 100141, China

However, there is still little information available on the use of medicinal plants for the treatment of *D. intermedius* infestation in fish. Generally, medicinal plants are more eco-friendly, biodegradable, and non-resistible when compared to chemical anthelmintic agents (Prakash and Rao 1997; Prabakar and Jebanesan 2004; Rahuman et al. 2008); an attempt has therefore been made under the present work to exploit the different solvent extracts of *D. crassirhizoma*, *K. scoparia*, and *P. tenuifolia* against *D. intermedius* (Monogenea) in goldfish by in vivo anthelmintic efficacy assay.

Materials and methods

Parasites and hosts

Healthy goldfish, with a body weight of 3.7±0.3 g, were obtained from a local fish farm (Xi'an, Shaanxi Province, China) and were cultured under laboratory conditions (oxygen content higher than 85% saturation, 24±1°C) for a week prior to infection. Seven days later, the healthy goldfish were cohabitated at a ratio of 20% with the ones infected with *D. intermedius* which were cultured in our laboratory. The parasitized fish were prepared according to the procedure described in our previous study (Wang et al. 2008). Concisely, this procedure includes collecting eggs, hatching eggs, and re-infection. After 21 days, ten fish were randomly selected, killed by spinal severance, and checked for the presence and intensity of parasites on the gills under a light microscope (Olympus BX41, Tokyo, Japan) at 10×4 magnification. Fish were chosen for the in vivo anthelmintic efficacy assay when the infection rate was 100%, and the mean number of parasites on the gills was 40–50 per fish.

Plant materials

Fresh plant materials from each of the selected species (see Table 1) were collected in 2010. The specimen identification was confirmed by Prof. X.L. He (Northwest A&F University, Shanxi, China), and voucher specimens have been deposited in the College of Life Science, Northwest A&F University, China. They were washed, cut into small pieces, and then dried in an oven at 50°C until completely dried. The dried plant materials were then powdered separately and reduced to fine powder using a strainer (30–40 mesh). The powdered sample was freeze-dried at −54°C to ensure desiccation.

Preparation of the extracts and stock solutions

Five samples of each selected species (50.00 g) were respectively extracted with petroleum ether, chloroform, ethyl acetate, acetone, and methanol for 48 h, 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) at 50°C. The resulting extracts of different plants were dissolved in dimethyl sulfoxide (DMSO) and diluted with distilled water to obtain 0.4 $g mL^{-1}$ (sample/solvent) of stocking solutions, which were used for the preparations of the desired concentrations for anthelmintic efficacy assay.

In vivo anthelmintic efficacy assay

Tests were conducted in 5-L glass tanks, each containing 2 L of the test solution water and five parasitized fish. The water pH ranged from 7.0 to 7.5, and dissolved oxygen was between 6.2 and 7.8 mg L^{-1} (72%–85% saturation). All tests were performed at 24±1°C. The concentrations of different series of five crude extracts of the plants were determined based on the initial tests. A control group without extracts was set up under the same experimental conditions as the test groups. Another control, containing the highest percentage of DMSO, was also included to exclude the possible effects of DMSO on the parasites. All the experiments were conducted twice. During the experiments, no food was offered to the fish. 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. The effectiveness of each treatment was confirmed by comparison of the number of parasites in each treatment group with that in the control group. Finally, anthelmintic efficacy of each treatment and control group was calculated using the follow equation (Wang et al. 2008):

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

where

- E Anthelmintic efficacy
- B Average number of surviving *D. intermedius* in the negative control

Table 1 Characterization of plant products used in anthelmintic efficacy tests against *D. intermedius*

Plant name	Family name	Test part	Origin
Dryopteris crassirhizoma	*Dryopteridaceae*	Rhizomes	Shaanxi, Hanzhong, China
Kochia scoparia	*Chenopodiaceae*	Fruits	Shaanxi, Ankang, China
Polygala tenuifolia	*Polygalaceae*	Roots	Shaanxi, Weinan, China

T Average number of surviving *D. intermedius* in the treatment groups

P Average number of surviving *D. intermedius* in the positive control

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. A 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

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

Results

In vivo anthelmintic efficacy assay

The anthelmintic efficacies, EC_{50} and EC_{90} values, of different extracts of *D. crassirhizoma*, *K. scoparia*, and *P. tenuifolia* were shown in Figs. 1 and 2. In the case of *D. crassirhizoma*, methanolic extract was found to be the most effective with EC_{50} and EC_{90} values of 22.97 and 33.10 mg L^{-1}; it exhibited a 100% efficacy against *D. intermedius* at 60.00 mg L^{-1}, followed by the acetone,

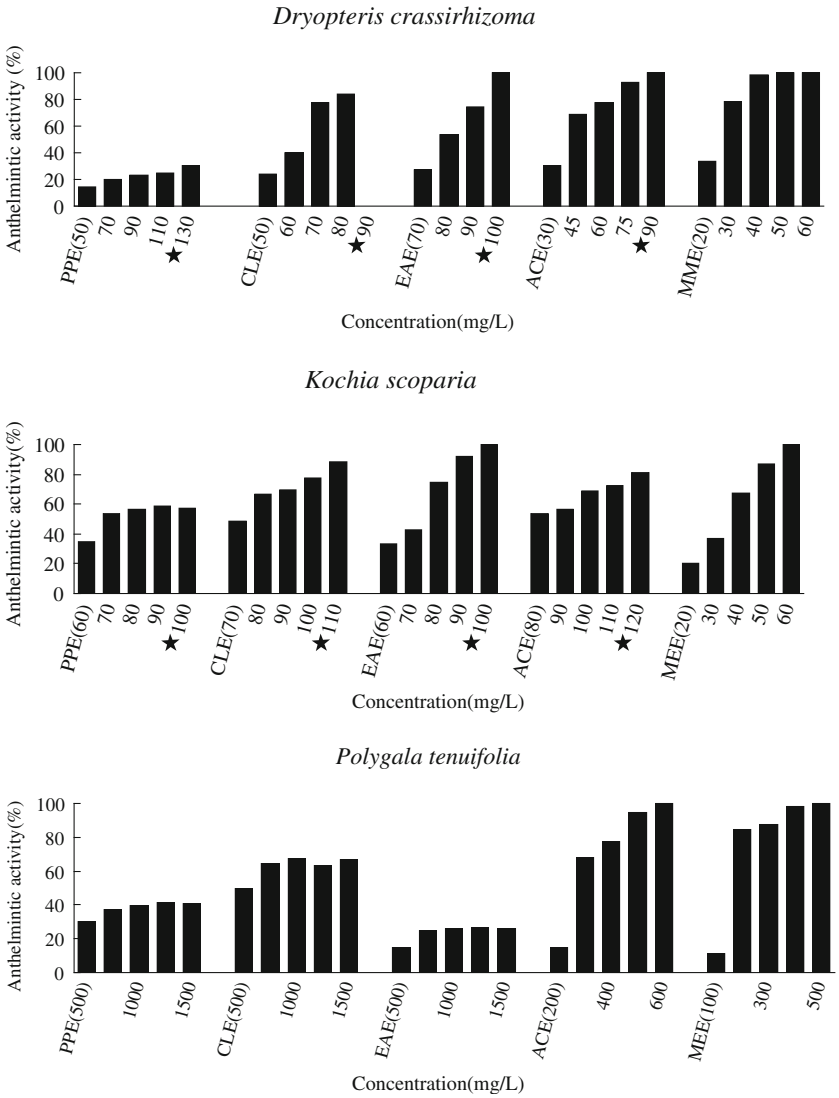

Fig. 1 Anthelmintic efficacy of different extracts of *D. crassirhizoma*, *K. scoparia*, and *Polygala* against *D. intermedius* after 48 h (*PEE* petroleum ether extract, *CLE* chloroform extract, *EAE* ethyl acetate extract, *ACE* acetate extract, *MEE* methanol extract; *star* indicates when fish mortality first occurred)

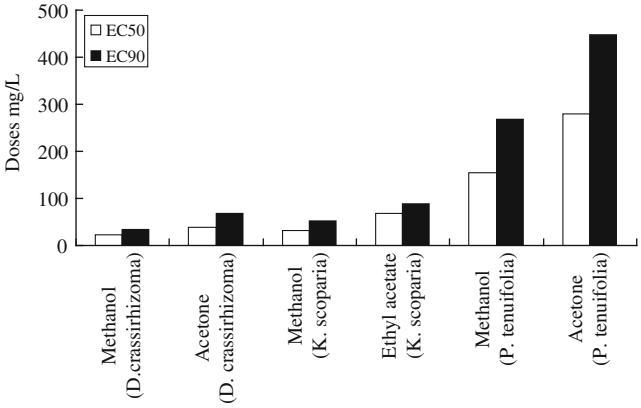

Fig. 2 EC_{50} and EC_{90} values of different extracts of *D. crassirhizoma*, *K. scoparia*, and *P. tenuifolia* against *D. intermedius* after 48 h

chloroform, and ethyl acetate extracts, with the maximum anthelmintic efficacies of 92.73% (95.00 mg L^{-1}), 83.79% (80.00 mg L^{-1}), and 74.73% (90.00 mg L^{-1}), respectively. EC_{50} and EC_{90} values for acetone and ethyl acetate extracts were 37.89 and 67.67 mg L^{-1}, 78.19 and 94.96 mg L^{-1}, accordingly. The petroleum ether extract was found to exhibit weak activity with the maximum anthelmintic efficacy of 24.67% at 110.00 mg L^{-1}.

In the case of *K. scoparia*, methanolic extract was observed to be the most effective with EC_{50} and EC_{90} values of 31.28 and 52.52 mg L^{-1}, while it exhibited a 100% efficacy against *D. intermedius* at 60.00 mg L^{-1}. The extract of ethyl acetate also showed high anthelmintic activity, with EC_{50} and EC_{90} values of 68.71 and 88.59 mg L^{-1}. However, fish mortality occurred when the concentration reached 100.00 mg L^{-1}, followed by the chloroform and the acetone extracts, which exhibited anthelmintic efficacies of 77.60% and 72.34% at 100.00 and 110.00 mg L^{-1}, respectively. Petroleum ether extract of *K. scoparia* exhibited the least activity with the maximum anthelmintic efficacy of 58.91% (90.00 mg L^{-1}).

As for *P. tenuifolia*, methanolic and acetone extracts displayed the optimal anthelmintic activity with 100% efficacy at the doses of 500.00 and 600.00 mg L^{-1}. EC_{50} and EC_{90} values were 154.78 and 267.79 mg L^{-1} for methanolic extract, 279.29 and 447.48 mg L^{-1} for acetone extract, accordingly. Chloroform, petroleum ether, and ethyl acetate extracts showed no obvious activity with the highest anthelmintic efficacy of 67.26%, 41.34%, and 26.76%, respectively.

Acute toxicity

The results of acute toxicity assay for methanolic extracts of *D. crassirhizoma*, *K. scoparia*, and *P. tenuifolia* can be seen in Fig. 3. The 48-h LC_{50} values of methanolic extracts of *D. crassirhizoma*, *K. scoparia*, and *P. tenuifolia* were 94.09, 71.04, and 774.31 mg L^{-1}; the LC_{90} values were 97.03, 78.01, and 801.20 mg L^{-1}, respectively.

Discussion

D. intermedius can cause serious economic damage in the aquaculture industry; the alarming spread of drug resistance and limited number of effective drugs now available underline how important it is to discover new anti-parasite agents. In the present work, an effort was made to investigate the efficacy of different extracts of *D. crassirhizoma*, *K. scoparia*, and *P. tenuifolia* against *D. intermedius*. The results indicated that methanolic extract of *D. crassirhizoma* was the most effective with EC_{50} value of 22.97 mg L^{-1}, followed by the methanolic extracts of *K. scoparia* and *P. tenuifolia* with EC_{50} values of 31.28 and 154.79 mg L^{-1}, respectively. As far as we know, it is the first study evaluating the extracts of *D. crassirhizoma*, *K. scoparia*, and *P. tenuifolia* against fish parasites.

D. crassirhizoma is a medicinal plant and recorded as Mianma Guanzhong in Chinese pharmacopoeia (The Pharmacopoeia Commission of the People's Republic of China, 2005). It has been used as a vermifuge, astringent, vulnerary, antibacterial, and anti-inflammatory agent, and used internally in the treatment of hemorrhage, uterine bleeding, and mumps (Bae 2000). Gao et al. (2002) reported that the methanolic extracts of *D. crassirhizoma* have antimalarial (*Plasmodium berghei)* activity with EC_{50} and EC_{90} values of 263.69 and 2,255.86 mg kg^{-1}. The major constituents, associated with the pharmacological activity of *D. crassirhizoma*, include phloroglucinol, triterpene, flavonoid, and other phenolic analogs (Min et al. 2001; Shiojima et al. 1990; Noro and Okuda 1973; Chang et al. 2006). Lee et al. (2009) pointed out that two phloroglucinols isolated from the methanolic extracts of *D. crassirhizoma* rhizomes were highly active against Gram-positive bacteria, such as methicillin-resistant *Staphylococcus aureus* KCTC 1928 (a MRSA bacterium), *Streptococcus mutans*, and *Bacillus subtilis*. In the case of triterpene, two triterpenes (2β-hydroxy-21βH-hop-22(29)-ene-24-oic acid, 2β-hydroxy-21βH-hop-22(29)-ene-23-oic

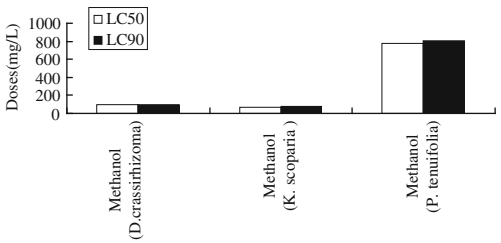

Fig. 3 LC_{50} and LC_{90} of methanolic extracts of *D. crassirhizoma*, *K. scoparia*, and *P. tenuifolia* against goldfish after 48 h

acid) isolated from the rhizome of *D. crassirhizoma* were found to have the potent ability of inhibiting HIV-1 protease with IC_{50} values of 8.9–44.5 μm(Lee et al. 2008); these substances may contribute to the antiparasitic properties independently or jointly and require further elucidation.

K. scoparia fruits is a well known Chinese medicine used to treat skin diseases (Matsuda et al. 1997a, b). Shin et al. (2004) found that the methanol extract of *K. scoparia* fruits can diminish inflammation by blocking nuclear factor-κB activation to inhibit lipopolysaccharide-induced inducible NO synthase and cyclooxygenase-2 expression. Momordin, the main glycosides contained in it, show antinociceptive, anti-inflammatory (Matsuda et al. 1997a), and antiallergic (Matsuda et al. 1997b) activities. Study on *K. scoparia* revealed that the main compounds were glycosides and steroids (China Pharmaceutical University et al. 1993). In China, the rhizome of *P. tenuifolia* has been used as an expectorant, tonic, and sedative agent. Nagai et al. (2001) reported that onjisaponins A, E, F, and G isolated from hot water extracts from the root of *P. tenuifolia* can provide safe and potent adjuvants for intranasal inoculation of influenza HA and diphtheria–pertussis–tetanus vaccines. Previous research on the chemical components of *P. tenuifolia* has proved the presence of onjisaponins A–G (Sakuma and Shoji 1981a, b), various xanthones (Ito et al. 1977; Ikeya et al. 1991), different alkaloids (Byung et al. 1985), and polygalitol (Takiura and Honda 1964). Although, in this study, the compounds which are responsible for the observed anthelmintic activity were not identified, some of the substances mentioned above are believed to have anthelmintic activity independently or jointly.

The results of acute toxicity assay for the methanolic extracts of *D. crassirhizoma*, *K. scoparia*, and *P. tenuifolia* indicated that these extracts were safe to goldfish. The 48-h LC_{50} values of these extracts were 4.10-, 2.27-, and 5.00-fold higher than the corresponding EC_{50}. The chemical composition contained in medicinal plants is complicated; some compounds may have no effect on *D. intermedius*; however, they are toxic to fish. Therefore, the active components may be safer to fish.

In summary, the methanolic extracts of *D. crassirhizoma*, *K. scoparia*, and *P. tenuifolia* all have the potential for the development of novel therapy for the treatment against *D. intermedius* infection. However, the active components responsible for the anthelmintic activity need to be further investigated. In addition, field studies of these extracts are required for the practice use in fish aquaculture.

Acknowledgments This work was supported by the National High Technology Research and Development Program of China (863 Program) (no. 2011AA10A216) and National Natural Science Foundation of China (no. 31072242).

References

Bae K (2000) The medicinal plants of Korea. Kyo-Hak Publishing Company, Seoul, p 25

Bagavan A, Rahuman AA, Kamaraj C, Kaushik NK, Mohanakrishnan D, Sahal D (2010) Antiplasmodial activity of botanical extracts against *Plasmodium falciparum*. Parasitol Res 108(5):1099–1109

Byung HH, Jeong HP, Myung HP, Yong NH (1985) β-Carboline alkaloids of *Polygala tenuifolia*. Arch Pharm Res 8(4):243–247

Chang XL, Li W, Koike K, Wu LJ, Nikaido T (2006) Phenolic constituents from the rhizomes of *Dryopteris crassirhizoma*. Chem Pharm Bull 54:748–750

China Pharmaceutical University et al (1993) Traditional Chinese medicine Cihai, vol I. Chinese Medical Technology Publishing House, Beijing, pp 1937–1939

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

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

Gao ZP, Lu WR, Li GF (2002) Screening of the antimalarial parts of the extract of Guanzhong (the rhizome of *Dryopteris crassirhizoma*). Journal of Beijing University of Traditional Chinese Medicine 25(4):46–46

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 Wildlife Dis 16 (3):343–346

Ikeya Y, Sugama K, Okada M, Mitsuhashi H (1991) Two xanthones from *Polygala tenuifolia*. Phytochemistry 30:2061–2065

İsmail KIR, Selda TÖ (2007) Helminth infections in common carp, *Cyprinus carpio* L., 1758 (Cyprinidae) from Kovada Lake (Turkey). Turk Parazitol Derg 31:232–236, in German with English abstract

Ito H, Taniguchi H, Kita T, Matsuki Y, Tachikawa E, Fujita T (1977) Xanthones and a cinnamic acid derivative from *Polygala tenuifolia*. Phytochemistry 16:1614–1616

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

Lee HB, Kim JC, Lee SM (2009) Antibacterial activity of two phloroglucinols, flavaspidic acids AB and PB, from *Dryopteris crassirhizoma*. Arch Pharm Res 32(5):655–659

Lee JS, Miyashiro H, Nakamura N, Hattori M (2008) Two new triterpenes from the rhizome of *Dryopteris crassirhizoma*, and inhibitory activities of its constituents on human immunodeficiency virus-1 protease. Chem Pharm Bull 56(5):711–714

Marshall CJ (1999) Use of Supaverm® for the treatment of monogenean infestation in koi carp (*Cyprinus carpio*). Fish Veterinary J 4:33–37

Matsuda H, Dai Y, Ido Y, Ko S, Yoshikawa M, Kubo M (1997a) Studies on kochiae fructus III. Antinociceptive and antiinflammatory effects of 70% ethanol extract and its component, momordin Ic from dried fruits of *Kochia scoparia* L. Biol Pharm Bull 20:1086–1091

Matsuda H, Dai Y, Ido Y, Ko S, Yoshikawa M, Kubo M (1997b) Studies on kochiae fructus IV. Anti-allergic effects of 70% ethanol extract and its component, momordin Ic from dried fruits of *Kochia scoparia* L. Biol Pharm Bull 20:1165–1170

Min BS, Tomiyama M, Ma CM, Nakamura N, Hattori M (2001) Kaempferol acetylrhamnosides from the rhizome of *Dryopteris crassirhizoma* and their inhibitory effects on three different activities of human immunodeficiency virus-1 reverse transcriptase. Chem Pharm Bull 49:546–550

Nagai T, Suzuki Y, Kiyohara H, Susa E, Kato T, Nagamine T, Hagiwara Y, Tamura SI, Yabe T (2001) Onjisaponins, from the root of *Polygala tenuifolia* Willdenow, as effective adjuvants for nasal influenza and diphtheria–pertussis–tetanus vaccines. Vaccine 19:4824–4834

Noro Y, Okuda K (1973) Dryocrassin: a new acylphloroglucinol from *Dryopteris crassirhizoma*. Phytochemistry 12:1491–1492

Prakash A, Rao J (1997) Botanical pesticides in agriculture. CRC Lewis, New York, p XIII

Prabakar K, Jebanesan A (2004) Larvicidal efficacy of some cucurbitacious plant leaf extracts against *Culex quinquefasciatus* (Say). Bioresour Technol 95(1):113–114

Rahuman AA, Gopalakrishnan G, Venkatesan P, Geetha K (2008) Larvicidal activity of some Euphorbiaceae plant extracts against *Aedes aegypti* and *Culex quinquefasciatus* (Diptera: Culicidae). Parasitol Res 102:867–873

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. Accessed 17 May 2009

Sakuma S, Shoji J (1981a) Studies on the constituents of the root of *Polygalae tenuifolia* Willdenow. Chem Pharm Bull 29:2431–2441

Sakuma S, Shoji J (1981b) Studies on the constituents of the root of *Polygalae tenuifolia* Willdenow. Chem Pharm Bull 30:810–821

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

Shin KM, Kim YH, Park WS, Kang I, Ha J, Choi JW, Park HJ, Lee KT (2004) Inhibition of methanol extract from the fruits of *Kochia scoparia* on lipopolysaccharide-induced nitric oxide, prostagladin E2, and tumor necrosis factor-a production from murine macrophage RAW 264.7 cells. Biol Pharm Bull 27 (4):538–543

Shiojima K, Arai Y, Ageta H (1990) Seasonal fluctuation of triterpenoid constituents from dried leaflets of *Dryopteris crassirhizoma*. Phytochemistry 29:1079–1082

Takiura K, Honda S (1964) Sugar components of the root of *Polygala tenuifolia*. Yakugaku Zasshi 84:1223–1224

The Pharmacopoeia Commission of People's Republic of China (2005) The pharmacopoeia of the People's Republic of China, vol. I. Chemical Industry Publishing House, Beijing, p 231

Topić PN, Hacmanjek M, Teskeredžić E (2001) Health status of rudd (*Scardinius erythrophthalmus hesperidicus* H.) in Lake Vrana on the Island of Cres, Croatia. J Appl Ichthyol 17:43–45

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

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