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ORIGINAL PAPER

Effects of aqueous extract of *Capsicum frutescens* (Solanaceae) against the fish ectoparasite *Ichthyophthirius multifiliis*

Fei Ling · Jian-Guo Wang · Cheng Lu · Gao-Xue Wang · Yong-Hui Lui · Xiao-Ning Gong

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Abstracts *Ichthyophthirius multifiliis* is an important fish ectoparasite that often results in significant economic losses to freshwater aquaculture. The search of alternative substances to control infections of *I. multifiliis* became stringent after malachite green, an effective and widely used chemotherapeutant, is banned on fish farms because of its carcinogenicity and teratogenicity. In this study, the effects of the aqueous extract of *Capsicum frutescens*, which is readily available and affordable, were evaluated under in vitro and in vivo conditions. The results in the in vitro conditions showed that the aqueous extracts of *C. frutescens* with the ratios (V_{SS}/V_T—V_{SS}, the volume of stock solution; V_T, the volume of total solution) of 1:32 and 1:64 led to more than 70 % mortality of *I. multifiliis* theronts during 4 h of exposure and significantly reduced the survival of the tomonts and the total number of theronts released by the tomonts within 22 h ($P<0.05$). A 96-h bioassay was carried out to determine the acute toxicity of the aqueous extract of *C. frutescens* to goldfish. No visible effect was observed in the treatments with the aqueous extracts of *C. frutescens* with the ratios (V_{SS}/V_T) of 1:32, 1:64 and 1:128, while in the other treatments, the erratic behaviour of fish was noted. In addition, in vitro tests demonstrated that the aqueous extract of *C. frutescens* had an adverse effect on *I. multifiliis* trophonts in situ. Fish treated with the aqueous extracts of *C. frutescens* in ratios V_{SS}/V_T of 1:32 and 1:64 carried significantly fewer parasites than the control and the other treatments ($P<0.05$). These results suggest, therefore, that aqueous extracts of *C. frutescens* have potential for the control of ichthyophthiriasis in the aquaculture industry, though further phytochemical studies will need to be performed for isolation and identification of the active compounds.

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Introduction

The ciliate *Ichthyophthirius multifiliis* Fouquet, 1876, commonly called "ich", is the main parasitic threat to freshwater fish in most climatic zones (Buchmann et al. 2001). The parasite, with a wide temperature tolerance (Wagner 1960; Aihua and Buchmann 2001) and a very low degree of host specificity (Buchmann and Nielsen 1999), is probably the most widespread parasite of freshwater teleosts with a geographical range extending from the tropics to temperate regions, northwards in Europe to the Arctic Circle (Matthews 2005), and caused disease (ichthyophthiriasis) not only in wild freshwater fish and in freshwater aquaculture (Nigrelli et al. 1976; Wurtsbaugh and Tapia 1988; Buchmann and Bresciani 1997; Traxler et al. 1998; Rintamaki-Kinnunen et al. 2005a,b), but also in the ornamental fish trade (Kim et al. 2002; Thilakaratne et al. 2003; Matthews 2005). Infections with *I. multifiliis* are causing extensive economic losses for conventional earth pond fish farmers as well as fish farmers using new high-technology re-circulated systems (Jorgensen et al. 2008; Heinecke and Buchmann 2009).

The life history of *I. multifiliis*, which consists of three stages: an infective theront, a parasitic trophont and a reproductive tomont, is well documented (Nigrelli et al. 1976; Noe and Dickerson 1995; Swennes et al. 2006). A free-

swimming theront infects the gills and skin surface of the fish to feed on mucus and tissue and rapidly differentiates into a trophont, the feeding stage, which resides within the epidermis of the fish. Following a period of growth and development, it leaves the host actively and transforms to an encysted tomont. The tomont undergoes mitosis in the cyst, and a large number of small tomites are produced and liberated as theronts, the stage infective to the fish host.

Formerly, ichthyophthiriasis was treated using malachite green and mixtures of malachite green and formalin by immersion with a good deal of success (Leteux and Meyer 1972; Wahli et al. 1993; Dickerson and Dawe 1995; Buchmann et al. 2003). However, the use of malachite green for the treatment of disease has been no longer permitted by some government agencies, such as the Food and Drug Administration of the USA, due to its potential carcinogenic and teratogenic properties (Alderman 1985; Srivastava et al. 2004). The effects of other chemicals, such as copper sulphate (Ling et al. 1993; Schlenk et al. 1998), bronopol (Shinn et al. 2001), sodium chloride (Selosse and Rowland 1990), chloramine-T (Cross and Hursey 1973), potassium permanganate (Straus and Griffin 2002) and potassium ferrate (Ling et al. 2010), have been evaluated as treatments. However, the application of chemical treatments in commercial aquaculture has to face many significant problems: low efficacy, high cost and environmental health concerns or are unlikely to receive regulatory approval (Dickerson and Dawe 1995; Tieman and Goodwin 2001; Ling et al. 2010).

Recently, increased research activities have been demonstrated on the utilization of plant extracts to treat parasitic diseases in animals, including fish (Madsen et al. 2000; Paolini et al. 2003; Zahir et al. 2009; Wang et al. 2009, 2010). Buchmann et al. (2003) reported raw extracts from garlic (*Allium sativum*) were used to treat *I. multifiliis* infestation by killing theronts and tomonts. Ekanem et al. (2004) accessed the effects of crude extracts from *Mucuna pruriens* and *Carica papaya* against *I. multifiliis*. Green tea extract has been reported to kill *Ichthyobodo necator* (Suzuki et al. 2006). *Capsicum frutescens*, as Mayan and Chinese medicine, is readily available and affordable. It has exhibited antibacterial and antivirus activities (McKeen 1956; Abdou et al. 1972; Cichewicz and Thorpe 1996). At present, little report has referred to the antiparasitic activity of extracts of *C. frutescens*. The aim of this study was to access the effects of aqueous extracts from *C. frutescens* against *I. multifiliis*, fish ectoparasite.

Materials and methods

Fish

Goldfish (*Carassius auratus*), weighing 3.87±0.91 g, were utilized throughout the study. All fish, referred to as 'naive fish', were kept in several 300-L opaque tanks and supplied with a constant flow of aerated tap water (flow rate, 1.0–1.5 L min^{-1}), with water temperature of 22.0±2.0 °C, pH of 7.1± 0.4 and dissolved oxygen of 5.0–7.1 mg/L. They were fed once at 1 % body weight daily with commercial fish pellet feed.

Parasite

A local strain of *I. multifiliis* was isolated from *Astronotus ocellatus*, obtained from a pet shop, and its passage was as Ling et al. (2009, 2010) described. The parasitized fish and healthy goldfish were held at 22±2 °C in a static 40-L aquarium equipped with an outside biological filter and air stones to maintain enough dissolved oxygen (greater than 5 mg/L). *I. multifiliis* was collected using a method described by Clayton and Price (1988). Several heavily infected fish were placed into 300 mL of filtered aquarium water for 30 min. Mature trophonts were allowed to dislodge from the host by body movements of the fish whilst in close proximity. The cysts thus obtained were incubated at 23.5±0.5 °C for 18–20 h, and theronts were allowed to emerge naturally. The infectious theronts were used to determine acute toxicity of test solutions to *I. multifiliis* and challenge fish during experiments. Theront concentrations were determined by pipetting 2-μL droplets of the theront suspension onto a glass slide and counting the organisms (×40 magnification); the mean count in ten droplets was extrapolated to determine the final concentration (Schlenk et al. 1998).

Aqueous extracts of *C. frutescens*

The aqueous extract of *C. frutescens* was prepared by decoction according to the method by Nalina and Rahim (2006). The dried fruits of *C. frutescens* were purchased from one market in Wuhan, China, and in this study, the fruits were obtained from the same source. Prior to decoction, *C. frutescens* fruits were cleaned, cut to small pieces and weighed. The pieces of *C. frutescens* fruits were put to boil in deionized distilled water in a ratio of 5 % *W*/*V* (*W*, weight of *C. frutescens* fruits; *V*, volume of deionized distilled water) for 20 min. The resulting mixture was then filtered through muslin cloth. The filtrate obtained was regarded as stock solution and was reconstituted in deionized distilled water to give the desired concentration of the test solution at a ratio of V_{SS}/V_T (V_{SS}, the volume of stock solution; V_T, the volume of total solution). Throughout this study, fresh aqueous extract of *C. frutescens* was used prior to each experiment.

In vitro tests

An in vitro experiment was designed to determine the acute toxicity of the aqueous extract of *C. frutescens* to *I.*

multifiliis theronts according to an immobilization method (Sin et al. 1991; Ling et al. 1993; Straus and Griffin 2001). Approximately 500 theronts were placed into each well of a 96-well microtitre plate and exposed to test solutions containing the aqueous extracts of *C. frutescens* with the ratios (V_{SS}/V_T) of 1:2, 1:4, 1:8, 1:16, 1:32, 1:64, 1:128, 1:256 and 1:512, respectively. Acute toxicity was assessed by microscopic examination of each well at various intervals up to 4 h after treatment (Ling et al. 2010). Mortality of theronts was determined by the absence of motility and abnormal morphology. The experiment was conducted at 23.5±0.5 °C and replicated three times using separate populations of theronts for each concentration of the aqueous extract of *C. frutescens*.

A toxicity assay was conducted to determine the effect of the aqueous extract of *C. frutescens* on the survival and reproduction of *I. multifiliis* tomonts according to an approach taken by Ling et al. (2011). One-hundred trophonts were distributed to each well of a 24-well tissue culture plate. After discarding the water in each well, 1 mL test solution containing the aqueous extracts of *C. frutescens* with the ratios (V_{SS}/V_T) of 1:4, 1:8, 1:16, 1:32, 1:64 and 1:128 was added to each well, respectively. The solutions were not changed throughout the experiment, and the 24-well plate with tomonts was incubated at 23.5±0.5 °C. At 22 h, the number of dead tomonts was counted under a dissecting microscope (×40 magnification). A dead tomont was determined by the absence of internal cell motility or abnormal cell division. Besides, theronts released by each tomont in each well were enumerated in ten 1- or 2-μL droplets of the theront suspension as Schlenk et al. (1998) described. The mortality and reproduction of tomonts were determined for each well according to Xu et al. (2008). The tomont reproduction was expressed as number of theronts released by each tomont, calculated by total theronts/live tomont. The experiment was repeated five times.

Toxicity tests of aqueous extracts of *C. frutescens* to goldfish

Aqueous static renewal 96-h bioassays were conducted to determine the acute toxicity of aqueous extracts of *C. frutescens* to goldfish (DeLorenzo et al. 2006; Ling et al. 2010). Goldfish were placed into several 20-L aquariums (ten fish/aquarium), and aqueous extracts of *C. frutescens* concentrations for goldfish aqueous exposures were with the ratios (V_{SS}/V_T) of 1:8, 1:16, 1:32, 1:64 and 1:128, respectively (a preliminary study was performed to establish a mortality range of 0–100 %). The test included a control without aqueous extracts of *C. frutescens*. The aquarium water was renewed every 24 h with fresh aqueous extract of *C. frutescens* or aerated tap water, and water quality parameters (dissolved oxygen, pH, temperature) were measured in all aquariums before the media were changed (DeLorenzo et al. 2006). Mortality observations were taken from each aquarium every day. All fish were not fed during the exposure (Buikema et al. 1980; DeLorenzo et al. 2006).

In vivo tests

In order to achieve consistent infestation of goldfish, the experiment was conducted to determine the appropriate number of infective theronts. Sixty healthy goldfish were divided into six groups (N=10) and exposed in opaque breakers to 0, 1,000, 2,500, 5,000, 10,000, and 15,000 theronts per fish, respectively. Infection protocol was referred to Ling et al. (2009, 2010). For each group, first, theronts were placed into an opaque 2-L breaker prior to infection, and the goldfish were transferred into the breaker at a density of one fish per 100 mL of aerated tap water. After fish and theronts remained in close contact for 30 min, during which time infection occurs (McCallum 1982), all the contents of each beaker were placed into a static 20-L aquarium, equipped with air stones and in which the fish had been previously acclimated for at least 1 week. The aquarium water was renewed on alternate days with aerated tap water. The experiment was terminated on the third day following exposure to theronts. All fish were anaesthetized as Clayton and Price (1988) described. The anaesthetic, benzocaine (ethyl p-aminobenzoate), made up in a primary solution of 1 g/100 mL 80 % alcohol, was added slowly until the fish lost all motor ability but not respiratory activity. Then the fish were examined under a dissection microscope, and the number of trophonts on the fish fin was scored. All infections were carried out at 22.0±2 °C. The experiments were conducted with two replicates. Table 1 showed that theront concentration of 5,000 per fish consistently led to infestation.

An in vivo test was adapted from the method of Ling et al. (2010) to access the aqueous extract of *C. frutescens* effective against *I. multifiliis* trophonts in situ. This experiment consisted of three replications. In each replication, a

Table 1 Effect of theront concentrations on the prevalence of ichthyophthiriasis for goldfish (N=10) following a 3-day exposure

Theront concentration (number/fish)	Prevalence of ichthyophthiriasis[a] (%)
0	0
1,000	20
2,500	65
5,000	100
10,000	100
15,000	100

[a] Prevalence of ichthyophthiriasis: no. of infected fish/total no. of fish

dose of theronts (5,000 theronts per fish) and ten goldfish were added into an opaque 2-L beaker containing 1,000 mL aerated tap water for 30 min, and all the contents of each beaker were placed into a static 20-L aquarium containing the aqueous extracts of *C. frutescens* with the ratios (V_{SS}/V_T) of 1:32, 1:64, 1:128 and 1:256, respectively. Daily observation was recorded for fish survival, and the number of trophonts on the fish fin was scored on the third day following exposure to theronts, after the fish was anaesthetized as described above. Each aquarium was equipped with air stones, in which the fish had been previously acclimated for at least 1 week, and the water was renewed on the third day with aerated tap water (after scoring).

Statistic analysis

All data in this study were analysed by version 13.0 of Statistical Product and Service Solutions. The Student–Newman–Keuls test (S–N–K) for multiple comparisons was used to determine significantly different prevalences of ichthyophthiriasis and infection levels on the third day after exposure to *I. multifiliis* theronts (α=0.05). Owing to the data of non-normal distribution, a natural logarithmic transformation was carried out.

Results

In vitro test

At prolonged exposure, high concentrations of the aqueous extracts of *C. frutescens* resulted in high mortalities of *I. multifiliis* theronts (Table 2). During the 4-h exposure period, the aqueous extracts of *C. frutescens* with the ratios (V_{SS}/V_T) of 1:16 and 1:32 killed more than 90 % of *I. multifiliis* theronts, while no dead parasite was observed in the wells containing the aqueous extracts of *C. frutescens* with the ratios (V_{SS}/V_T) of 1:256 and 1:512, as well as in the controls. In this test, it is found that the theronts in the aqueous extracts of *C. frutescens* with the ratios (V_{SS}/V_T) of 1:16 or more were gradually changed into spherical shapes and congregated into grape-like aggregations after 1 h of exposure. Furthermore, some theronts also had a spherical appearance by 1 h of co-culture with the aqueous extracts of *C. frutescens* with the ratios (V_{SS}/V_T) of 1:32 and 1:64, but they were able to move, while no normal morphology or behaviour was seen in the other treatments (Fig. 1).

Table 3 shows the effects of the aqueous extract of *C. frutescens* on *I. multifiliis* tomont survival and reproduction: increasing concentrations of the extracts were associated with increased mortalities of tomonts, and no significant difference was noted on reproduction between treatments and the control, except the treatment in highest concentration of the aqueous extract of *C. frutescens*. More than 50 % of *I. multifiliis* tomonts were dead after 22 h of exposure to the aqueous extracts of *C. frutescens* with the ratios (V_{SS}/V_T) of 1:32 or more, although about 8 % mortality occurred in the control. During microscopic examination, dead tomonts were distinctly apparent than live tomonts (Fig. 2), and no signs of division and no cilia movement were noted; in contrast, most live tomonts could divide and release theronts. Besides, it was observed that the treatment with the aqueous extract of *C. frutescens* resulted in a distinct dose-dependent decrease in the total number of *I. multifiliis* released by tomonts compared to the controls.

Toxicity tests to goldfish

The results of toxicity tests of the aqueous extract of *C. frutescens* to goldfish show that no visible effect was observed in the treatments with the ratios (V_{SS}/V_T) of 1:32, 1:64 and 1:128, though there was a dead fish found in one treatment for 96 h of exposure to the aqueous extract with the ratios (V_{SS}/V_T) of 1:32 (Table 4). The aqueous extracts of *C. frutescens* with

Table 2 Acute toxicity of the aqueous extracts of *C. frutescens* against *I. multifiliis* theronts in vitro

Final concentration (V_{SS}/V_T)	Percent mortality			
	30 min	1 h	2 h	4 h
Control	0 (0)	0 (0)	0 (0)	0 (0)
1:512	0 (0)	0 (0)	0 (0)	0 (0)
1:256	0 (0)	0 (0)	0 (0)	0 (0)
1:128	0 (0)	0 (0)	32 (96.33±13.65)	38 (115.00±17.00)
1:64	16 (46.67±15.63)	27 (81.00±11.53)	51 (154.00±5.57)	76 (227.67±18.14)
1:32	43 (128.33±18.14)	67 (199.67±12.06)	80 (241.33±16.86)	93 (278.00±10.54)
1:16	86 (258.33±9.01)	100 (−)	100 (−)	100 (−)
1:8	100 (−)	100 (−)	100 (−)	100 (−)
1:4	100 (−)	100 (−)	100 (−)	100 (−)
1:2	100 (−)	100 (−)	100 (−)	100 (−)

The data in parentheses mean number of dead theronts and were expressed as mean±SD of three replicates

− no live theront was found, V_{SS} the volume of stock solution, V_T the total volume

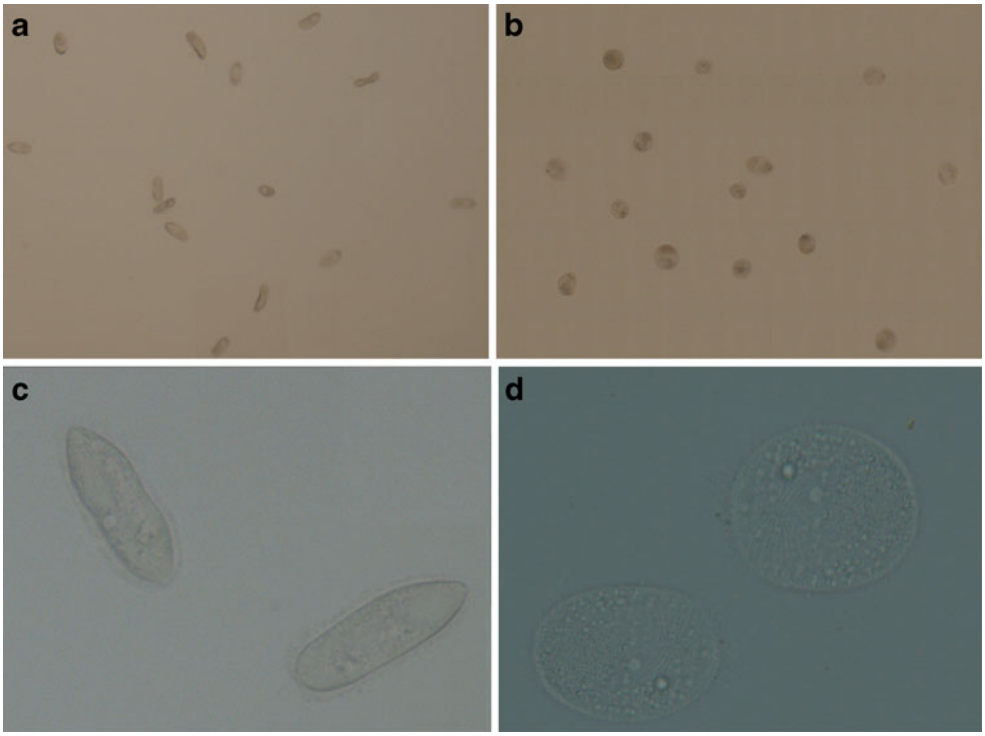

Fig. 1 Images of *I. multifiliis* theronts. **a** and **c** Theronts in the control (**a** ×100 magnification; **c** ×1,000 magnification). **b** and **d** Theronts after exposure to aqueous extract of *C. frutescens* (**a** ×100 magnification; **c** ×1,000 magnification)

the ratios (V_{SS}/V_T) of 1:16 and 1:8 were poorly tolerated and resulted in the erratic behaviour (agitated movement, an increased respiration frequency) of most fish within 24 h or before death, while the fish did not show any abnormal behaviour for 96 h in the controls (Table 4).

In vivo tests

A bath treatment with the aqueous extracts of *C. frutescens* with the ratios (V_{SS}/V_T) of 1:32, 1:64 and 1:128 resulted in a significant reduction of prevalence of ichthyophthiriasis compared to the controls (Table 5). On the third day after exposure, *I. multifiliis* trophonts were found in all test concentrations, as well as in the control. Fish treated with the aqueous extracts of *C. frutescens* with the ratios (V_{SS}/V_T) of 1:32 and 1:64 carried significantly fewer parasites than the control and the other treatments ($P<0.05$) (Table 5).

Table 3 Effect of the aqueous extracts of *C. frutescens* on *I. multifiliis* tomont survival and reproduction

Concentration (V_{SS}/V_T)	Tomonts		Theronts	
	Number of dead tomonts	Survival (%)	Number (×1,000)	Theronts per tomont
Control	8.00±2.65	92.00±2.65a	34.63±1.85a	376.21±9.63a
1:128	15.67±2.08	84.33±2.08b	26.26±0.50b	311.46±3.26a
1:64	30.67±1.53	69.33±1.53c	19.80±1.05c	285.43±9.94a
1:32	58.00±4.58	42.00±4.58d	11.40±0.89d	272.22±15.59a
1:16	81.33±2.52	18.67±2.52e	5.64±0.57e	303.00±13.41a
1:8	98.33±2.89	1.67±2.89f	0.55±0.94f	327.20a[a]
1:4	0	0f	0f	0b

Each value was expressed as mean±SD of three replicates, and within a column, the values followed by a different lowercase letter were significantly different ($P<0.05$)

V_{SS} the volume of stock solution, V_T the total volume

[a] There are live theronts in one replicate

Discussion

The disease ichthyophthiriasis, or 'white spot', caused by a parasitic ciliate, *I. multifiliis*, probably accounts for more damage to freshwater fish populations worldwide than any other eukaryote pathogen (Hines and Spira 1973; Rogers and Gaines 1975; Matthews 2005). Consequently, the search for an effective treatment for ichthyophthiriasis becomes stringent, after an effective and widely used chemotherapeutant (malachite green) against *I. multifiliis* infections is no longer permitted by legislation to be used in food fish. In this study, the results of in vitro tests have shown that the aqueous extract of *C. frutescens* led to a distinct decrease in the number of *I. multifiliis* theronts and tomonts. Besides, a bath treatment with the aqueous extracts of *C. frutescens* has a detrimental effect on *I. multifiliis* trophonts in situ. Therefore, the aqueous extract of *C. frutescens*, which is readily available and affordable, demonstrates the application potential as a therapeutic agent for external protozoan parasite infections.

The treatment aimed at interrupting the life cycle by killing the free-living stages of the parasite is considered

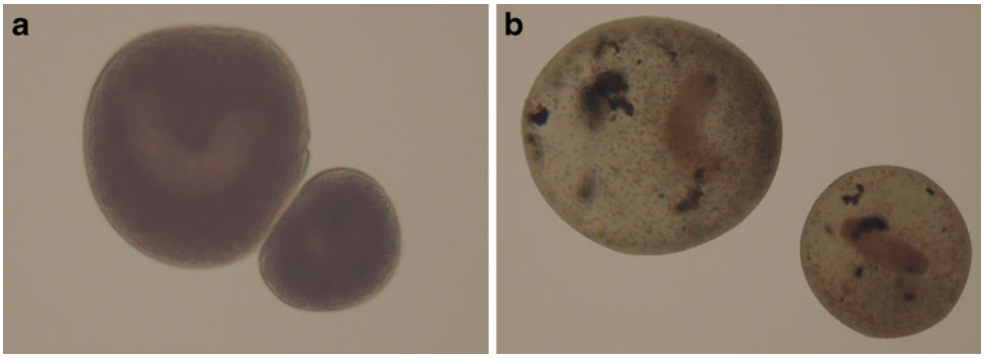

Fig. 2 Images of *I. multifiliis* tomonts. **a** Tomonts in the control (×400 magnification). **b** Tomonts after exposure to aqueous extract of *C. frutescens* (×400 magnification)

as an effective means of controlling the infections (Matthews 2005). In vitro tests in this study were performed to evaluate the susceptibility of *I. multifiliis* theronts and tomonts to the aqueous extract of *C. frutescens*. The results showed that the aqueous extracts of *C. frutescens* at a ratio (V_{SS}/V_T) of 1:64 can kill about 76 % of *I. multifiliis* theronts after 4 h of exposure (Table 2) and significantly reduce the survival of the tomonts and the total number of theronts released by the tomonts ($P<0.05$) (Table 3). In addition, it was observed that at this concentration, the aqueous extract of *C. frutescens* can result in more than 95 % mortality of *I. multifiliis* theronts within 24 h (data not shown). The result indicates that with the same concentration of the applied substances, increasing the length of treatment can obtain high parasite mortalities. Therefore, it is suggested that the aqueous extract of *C. frutescens* is used to prevent progressive invasion of the fish and the spread of the disease.

Also, the present in vivo work shows that the aqueous extract of *C. frutescens* significantly reduced the prevalence of ichthyophthiriasis and the number of trophonts on fish fins on day 3, though this extract was not given during the initial infection (30 min). McCallum (1982) considered that the infection was completed within the initial 30-min exposure of *I. multifiliis* theronts; in addition, it was concluded that the parasite burden of a fish resulted from the number of theronts to which it was exposed. It is assumed that the distinct reduction of the prevalence of ichthyophthiriasis and the number of trophonts on fish fins could be attributed to direct impacts on encysted trophonts and the further development of *I. multifiliis*. This finding indicates that the aqueous extract of *C. frutescens* is possibly effective to the feeding stage of this parasite. However, it was observed that the aqueous extracts of *C. frutescens* had no effects on reproduction of *I. multifiliis* tomonts, except the treatment at the highest concentration. We considered that under adverse circumstances, the reproductive potential of tomonts could be related to whether the tomont finished developing the cyst wall. Matthews (2005) and Meinelt et al. (2009) demonstrated that *I. multifiliis* tomonts encysted within 15 min to 6 h of leaving the host epidermis. In this in vitro test, it is found that the death of tomonts occurred within 6 h, and after 6 h of exposure, no visible effect was observed in the other living tomonts, compared with the tomonts in the control.

From the results of the toxicity tests, the aqueous extracts of *C. frutescens* showed early lethal effects on fish with the ratios (V_{SS}/V_T) of 1:8 and 1:16, but at a ratio (V_{SS}/V_T) of 1:32, a fish died at the end of the exposure period (96 h). Any abnormal behaviour was not noted in the treatment, and in the in vitro tests, no fish died during 96 h. Consequently, it is considered that the aqueous extracts of *C. frutescens* with the ratios V_{SS}/V_T of 1:32 or less were safe to goldfish.

Table 4 Acute toxicity of the aqueous extract of *C. frutescens* to goldfish in aqueous static renewal 96-h bioassays

Concentration (V_{SS}/V_T)	Total no. tested	No. dead				Survival (%)
		24 h	48 h	72 h	96 h	
Control	30	0	0	0	0	100
1:128	30	0	0	0	0	100
1:64	30	0	0	0	0	100
1:32	30	0	0	0	1	97
1:16	30	19	4	1	0	20
1:8	30	28	2	0	0	0

V_{SS} the volume of stock solution, V_T the total volume

Table 5 Effect of the aqueous extract of *C. frutescens* on the prevalence of ichthyophthiriasis and mean number of trophonts on the fins of each infected goldfish on day 3

Concentration (V_{SS}/V_T)	Prevalence of ichthyophthiriasis (%)	Mean number of trophonts per infected fish (no. infected)
Control (0)	100.00±0a	17.77±8.03a (30)
1:256	100.00±0a	17.63±7.02a (30)
1:128	80.00±10.00b	16.21±6.11a (24)
1:64	40.00±10.00c	5.58±3.02b (12)
1:32	13.33±11.55d	4.50±2.38b (4)

Each value was expressed as mean±SD of three replicates, and within a column, the values followed by a different lowercase letter were significantly different ($P<0.05$)

V_{SS} the volume of stock solution, V_T the total volume

C. frutescens, used for culinary purposes and as a traditional medicine, are widely cultivated in tropical and subtropical countries (Ivbijaro and Agbaje 1986; Rehm and Espig 1991). It has been investigated for antimicrobial properties: Abdou et al. (1972) reported that the crude juices of *C. frutescens* were active on *Escherichia coli*, *Salmonella typhi* and *Bacillus subtilis*. The plain and heated aqueous extracts from fresh *C. frutescens* were found to exhibit varying degrees of inhibition against five bacterial species (Cichewicz and Thorpe 1996). The results reported in this study have demonstrated that the aqueous extract of *C. frutescens* has potential for the control of parasitic diseases in cultures fish. However, the extracts still have to be evaluated under field conditions, and toxicity tests to other fish species also need to be accessed. Future studies will investigate on the isolation and characterization of the active compounds in this aqueous extract.

In conclusion, the results showed that the aqueous extracts of *C. frutescens* with the ratios (V_{SS}/V_T) of 1:32 and 1:64 led to more than 70 % mortality of *I. multifiliis* theronts by 4 h and significantly reduced the survival of the tomonts. Aqueous static renewal 96-h bioassays indicated that goldfish could tolerate the aqueous extracts of *C. frutescens* with the ratios (V_{SS}/V_T) of 1:32 or less. Additionally, the in vivo test demonstrated that a bath treatment with the aqueous extract of *C. frutescens* has a detrimental effect on *I. multifiliis* trophonts in situ.

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