Effects of mixed extract of thyme, mint and ginger on *Ichthyophthirius multifiliis* theronts: *In vitro* study

Rahmati-Holasoo H.[1*]; Nassiri A.[1]

Received: June 2024 Accepted: October 2024

Abstract

Ichthyophthiriasis is caused by the parasite *Ichthyophthirius multifiliis* (Ich). Certain chemical drugs are used as treatments for this parasite. However, given the myriad of detrimental impacts that chemicals have on the environment and people, it is crucial to conduct in-depth research on the efficacy of plant extracts in treating this parasite. This investigation assessed a mixed extract of thyme, mint and ginger in vitro antiparasitic activity against the parasite *I. multifiliis*. The anti-parasitic activity of solution composed of thyme, mint, and ginger extract against *I. multifiliis* was assessed in a lab setting following a 180-minute exposure at dosages ranging from 0.06 to 8 mL/l. A statistical comparison was made between the obtained data and the outcomes of the negative control treatment and the positive control sample (15 ppm formaldehyde). The dose of 8 and 4 mL/liter corresponds with the lowest time to eradicate more than 80% of parasites. This concentration outperformed formaldehyde, destroyed all of the theronts after 45 minutes, and showed a notable difference from formaldehyde. Higher doses and longer exposure times of the mixed extract can decrease the number of ICH theronts and are appropriate for controlling Ichthyophthiriasis.

Keywords: Antiparasitic effect, Herbal ingredients, Ichthyophthiriasis, ICH, *Ichthyophthirius multifiliis*

1- Department of Aquatic Animal Health, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran

*Corresponding author's Email: rahmatih@ut.ac.ir

Introduction

Aquaculture has emerged as a rapidly developing food production technology on a global scale. Within this growing field, the subsector of breeding and cultivating aquatic animals has also becoming a well-established economic activity (Seyrafi *et al*., 2009; Rahmati-Holasoo *et al*., 2010, 2020; 2021a; Sayrafi *et al*., 2011; Akbari *et al*., 2014; Sadeghinezhad *et al*., 2015; Sasani *et al*., 2016; Ahmadivand *et al*., 2016, 2020). *Ichthyophthirius multifiliis* is the obligatory holotrich parasite that causes Ichthyophthiriasis. This parasite belongs to the family *Ichthyophthrididae*, the order *Heminostomatida*, the class *Oligohymenophora*, and the *Hymenostomata* subclass (Canella *et al*., 1976; Corliss, 1976; Wright and Lynn 1995; Lin *et al*., 1996; Van Den Bussche *et al*., 2000; Rahmati-Holasoo *et al*., 2024b). All ages of freshwater fish, both farmed and wild, are susceptible to infection by this parasite, which can result in up to 100% death depending on the degree of contamination. Ichthyophthiriasis, also known as "white spot disease" because the primary symptom of the illness is the development of white spots on the host's body, can spread widely due to the displacement of the infected host, its wide host range, and its ability to multiply rapidly (Wurtsbaugh and Tapia, 1988; Aguilar *et al*., 2005; Matthews, 2005; Maceda-Veiga *et al*., 2009; Forwood *et al*., 2015; Larcombe *et al.*, 2024). This parasite is one of the most important infectious agents in the ornamental and edible fish industry (especially salmon), and it causes significant harm to the aquaculture industry globally each year (Rintamäki-Kinnunen and Valtonen, 1997). This parasite has three stages in its life cycle, which are theront, trophont, and tomont, in that order: the first stage swims freely in the water, the second stage is on the fish's body, and the third stage is the parasite's reproductive form (Matthews, 2005). Treatment for this disease is difficult because of the parasite's environment adaptability, temperature dependence throughout its life cycle, hiding place in the host's epithelium (where it hides from potentially lethal substances and medications), and formation of cysts around the tomont (Matthews, 2005; Dickerson, 2006). The initial stage of this parasite's existence, known as the infectious theront, is thought to be the most susceptible to therapy (Matthews, 2005). The respiratory and excretory systems of fish are affected by this parasite, which also targets the epithelium of their skin and gills (Hines and Spira, 1973).

To date, a number of chemical substances have been developed to treat the parasite *I. multifiliis*. Among these, formaldehyde, chloramine-T, green malachite, and copper sulfate might be included (Dickerson, 2006). All of these, though, have disadvantages. For instance, green malachite has been outlawed because of its carcinogenic properties and tendency to accumulate in fish tissue, even though it has great solubility in water and affects this parasite at all stages of life (Hines and Spira, 1973; Buchmann *et al*., 2001, Tieman and Goodwin, 2001). For best results, formaldehyde treatment should also be performed often. Furthermore, formaldehyde lowers the amount of oxygen in the air. Fish exposed to copper sulfate regularly may also become poisonous

(Murray and Peele, 2005). Fish epithelial tissue can be harmed by high dosages of chloramine-T, hence caution should be exercised when using it. Chemical treatments for this parasite have the potential to harm the environment and endanger public health. To combat this parasite, there is a pressing need for safe and effective substitute agents. Catechol compounds as dual-targeting agents for fish protection against *Ichthyophthirius multifiliis* infections have been studied (Qu *et al.*, 2024). Consequently, plant compounds can be an excellent substitute for chemical compounds because of their natural degradability, lack of drug resistance, and high effectiveness (Sanabria *et al*., 2009).

In vitro, garlic extract (*Allium sativum L.*) showed encouraging results against this parasite (Buchmann *et al*., 2003). Papaya seeds (*Carica papaya*), green velvet bean leaves (*Mucuna pruriens*) (Ekanem *et al*., 2004), *Maceleaya cordat*a extract (Yao *et al*., 2010), garlic and chamomile extract combination (Gholipour-Kanani *et al.*, 2012), aqueous extract of *Capsicum frutescens* (Ling *et al*., 2012), ethanolic extract of *Magnolia officinalis* and *Sophora alopecuroides* (Yi *et al*., 2012), methanolic extract of *Psoralea corylifolia* (Ling *et al*., 2013), and *Morus alba* acetone and ethyl acetate extract (Fu *et al*., 2014a) are some of the plant extracts that are effective against the parasite. Additionally, compounds extracted from plant extracts, including dihydrosanguinarine and dihydrochelerythrine (Yao *et al*., 2011), pentagalloylglucose (Zhang *et al*., 2013), chelerythrine and chloroxylonine (Shan *et al*., 2014), cynatratoside-C (Fu *et al*., 2014b), and 10-gingerol (Fu *et al*., 2019), were effective against this parasite too. Studies conducted in Iran have examined the effects of tannic acid, pomegranate peel alcoholic extract, *Zataria multiflora*, and *Chelidonium majus L.* alcoholic extract on *Ichthyophthirius multifiliis* (Alavinia *et al*., 2018; Rahmati-Holasoo *et al*., 2021b; Yazdani Anaraki *et al*. 2021; Alijanpour *et al*., 2022; Rahmati-Holasoo *et al*., 2024a).

A perennial herb, thyme (*Thymus vulgaris*) is a member of the *Lamiaceae* mint family. It is native to the Mediterranean region and is distinguished by its robust stem and small, scented leaves. Thyme is highly valued for its strong and pleasant aroma and is commonly used as a culinary herb. But its importance goes far beyond the kitchen-for ages, it has been used in medicine. This is because active ingredients such as carvacrol and thymol exist and have significant antibacterial, antioxidant, and anti-inflammatory properties. Thyme has been used historically to cure a variety of illnesses, including digestive and respiratory problems. According to scientific studies, thyme shows efficacy against a variety of parasites. Notably, studies have demonstrated that thyme possesses antiprotozoal properties against a variety of protozoan parasites, including *Entamoeba histolytica*, *Giardia lamblia*, *Trichomonas vaginalis*, and *Toxoplasma gondii*. Certain components of thyme extract have demonstrated significant efficacy against certain parasites. Additionally, thyme can kill larvae due to its larvicidal properties. This comprises *Anisakis* larvae, which in humans can result in anisakiasis. Its ability

to cure rabbit hepatic coccidiosis has also been investigated (Dardona *et al*., 2024).

Zingiber officinale is the scientific name for the ginger plant, which is grown in Asia and has long been used in Europe. Ginger is said to be effective because of its carminative, fragrant, and absorbent qualities. Aside from its anti-inflammatory and anti-cholesterolaemic effects, ginger also has anti-arthritic, anti-migraine, anti-thrombotic, anti-inflammatory, hypolipidaemic, anti-nausea, anti-diabetic, antipyretic, antimicrobial, antischistosomal, antioxidant, hepatoprotective, diuretic, hypotensive, and gastrointestinal prokinetic activities. *Z. officinale* has been shown in prior research to exhibit strong anthelmintic activity against different strains of *Giardia lamblia*, *Blastocystis*, *Trypanosoma brucei*, *Toxocara canis*, *Angiostrongylus cantonensis*, *Dirofilaria immitis*, *Hymenolepis nana*, *Schistosoma mansoni*, and *Anisakis simplex*. The effectiveness of ginger solution as a bath and oral treatment for several parasites such as *Dactylogyrus sp*. in fish was investigated (Forouzan *et al*., 2012;Van *et al*., 2021).

It has been suggested that *Mentha piperita*, an aromatic member of the Lamiaceae family well-known in the culinary, medicinal, and pharmaceutical sectors, be included in fish diets. Antiseptic, antispasmodic, analgesic, anti-inflammatory, carminative, antifungal, antibacterial, anti-protozoan, and anti-helminthic qualities are among the many benefits of peppermint. Menthone and menthol are among the several chemical components found in *M. piperita* essential oil (Malheiros *et al*., 2016). A mixed extract (primarily composed of thyme, mint and ginger) was used in the present study. This research aims to study the antiparasitic effects of this substance on the ICH parasite in laboratory conditions.

Material and methods

Isolation of the parasite and preparation of parasite theronts

Initially, ten fish were bought from an ornamental fish store that were infected with the parasite *I. multifiliis*. The fish body mucus infected with adult trophonts was carefully used to create a wet smear, which was then carefully transferred to a petri dish filled with aquarium water to isolate the trophonts. The trophonts were then released into the water via pipetting. Under a microscope, infectious theronts with rapid motions were seen after three to five days at room temperature, or 25°C. Additionally, using a centrifuge (at 4000 rpm for 10 minutes), the density of theronts was attained at 6000 per milliliter. Theronts were also counted using a Thomas slide (Ling *et al*., 2013).

Preparation of extract concentrations

Eight distinct concentrations of a solution (primarily composed of thyme, mint and ginger extract) (0.06, 0.125, 0.25, 0.5, 1, 2, 4 and 8 mL/L), formaldehyde concentration (15 mg/L, positive control), and a negative control group without disinfectant solution (containing chlorine-free aquarium water) were prepared following preliminary experiments and a review of sources:

Treatment 1: negative control containing chlorine-free aquarium water

Treatment2: positive control containing formalin with a concentration of 15 mg/liter

Treatment 3: concentration of 8 mL/liter extract
Treatment 4: concentration of 4 mL/liter extract
Treatment 5: concentration of 2 mL/liter extract
Treatment 6: concentration of 1 mL/liter extract
Treatment 7: concentration of 0.5 mL/liter extract
Treatment 8: concentration of 0.25 mL/liter extract
Treatment 9: concentration of 0.125 mL/liter extract
Treatment 10: concentration of 0.06 mL/liter extract

Conditions of exposure of the theronts to different concentrations

First, 50 microliters, or 600 theronts, of theront suspension were given to each group. The solution (composed of thyme, mint, and ginger extract) was then administered to the parasite-containing wells in 50 microliter volumes at different concentrations using the doses specified in the preceding section. Following exposure, the parasite death rate was then calculated at 45, 90, and 180 minutes.

Calculating the survival and mortality rates of theronts

The number of deaths following the theronts' exposure period was determined using the following method: To sum up, 10 microliters of the contents of each well were placed under the coverslip and on the Thomas slide. The number of live theronts per cubic millimeter was then calculated by adding 10% to the total number of theronts that had been counted in nine big squares on the Thomas slide. The death of the theronts was ascertained by observing their metamorphosis, which is induced by cell lysis.

Statistical analysis

To calculate the effect of the solution on the parasite, the data from each experiment was analyzed using one-way ANOVA and Duncan's test ($p \leq 0.05$) in SPSS software (version 25) (Finney, 1971)

Results

Figure 1 shows the results of the laboratory investigation of the disinfectant solution at different concentrations (0.06–8 milliliters per liter) at different test times (45, 90, and 180 minutes) on the mortality rate of *Ichthyophthirius multifiliis* parasites. The results of the study showed that the death rate of parasitic theronts has a direct and significant relationship with the concentration of the compound and exposure time.

According to Figure 2, the shortest period to destroy more than 80% of parasites is related to 4 and 8 milliliters per liter, which were able to destroy all the parasites in 45 minutes. After 90 minutes, the concentration of 2 mL/liter was able to kill more than 90% of the parasites (Fig. 3), but after 180 minutes, it did not kill all the parasites (it killed 98% of the parasites after 180 minutes). However, the performance of this concentration after 180 minutes with doses of 4 and 8 mL/liter has no significant difference (Fig. 4), but at 45 and 90 minutes, there is a significant difference between the performance of this dose and the doses of 4 and 8 mL/liter. There is a significant difference between the

performance of the dose of 1 mL/liter at different times, so that in 45 minutes after exposure to the drug, it had no effect on the theronts, and in this respect, with its lower doses (0.5 to 0.06 milliliters/liter) has no significant difference. After 90 minutes, it was able to destroy 25%, and after 180 minutes, 56% of the parasites. Doses of 0.06 and 0.125 mL/liter, even after 180 minutes, had no effect on the reduction of the parasite population and did not record a significant difference with the negative control group.

Doses of 8 and 4 milliliters/liter after 45 minutes destroyed 100% of theronts, performed better than formaldehyde, and had a significant difference with formalin. The dose of 2 mL/liter after 90 minutes caused the disappearance of more than 80% of the parasites, and it was not significantly different from formalin, but it was significantly different from higher doses. The doses of 8, 4, and 2 mL/liter and formalin did not differ significantly after 180 minutes.

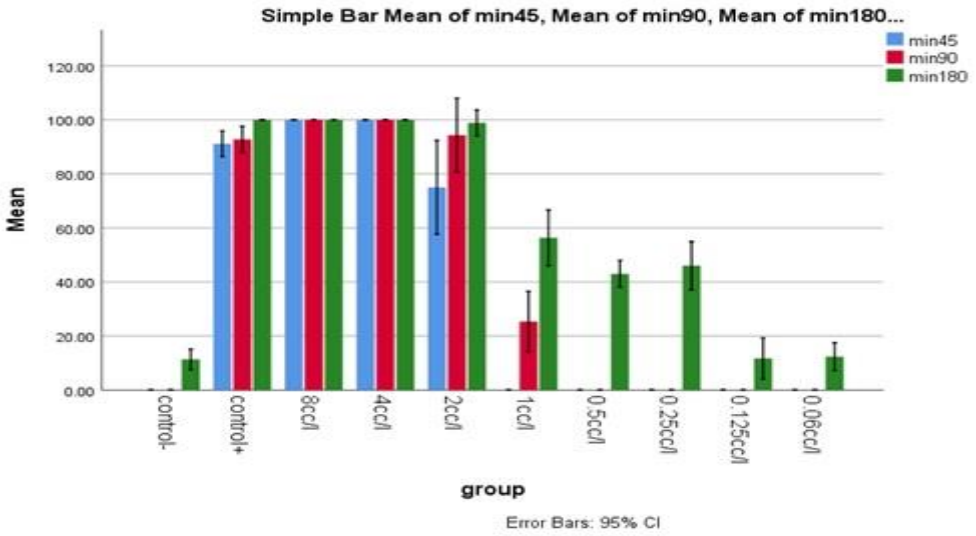

Figure 1: Mortality percentage of *Ichthyophthirius multifiliis* parasite as a function of mixed extract concentration (0.06-8 mL/l) and exposure time 45 to 180 minutes, formalin 15 ppm, and aquarium water without any solution as positive control treatments, and the negative control was considered.

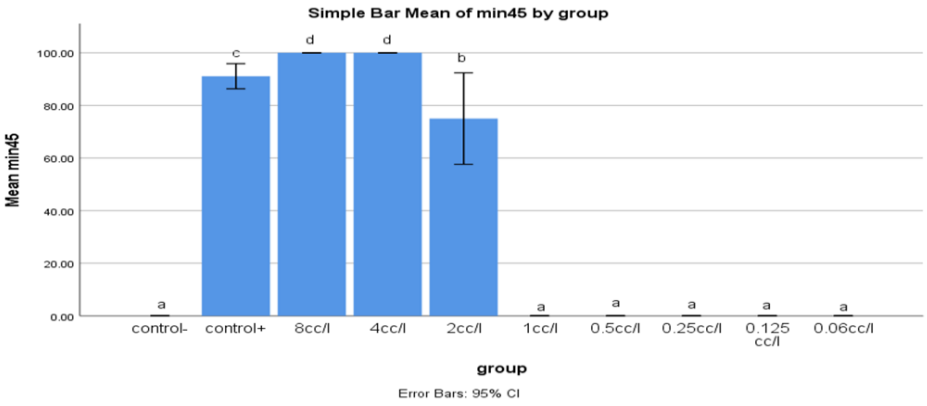

Figure 2: The percentage of mortality of *Ichthyophthirius multifiliis* theronts as a function of the different concentrations of the mixed extract (0.06-8 mL/l) and exposure time of 45 minutes. Formaldehyde (15 ppm) and aquarium water without any solution were considered positive and control treatments, respectively. Different statistical letters in each column indicate significant differences.

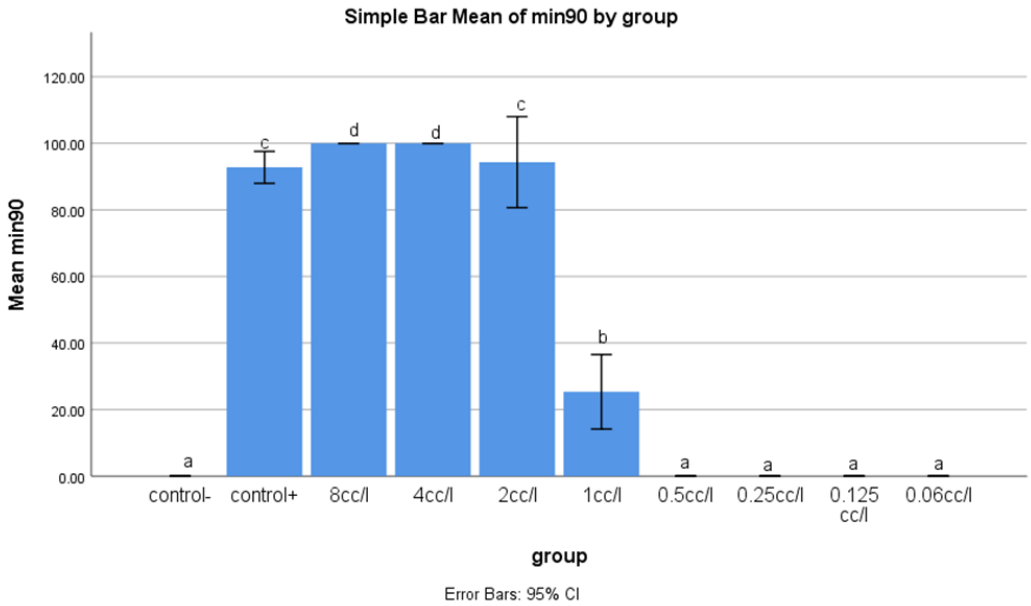

Figure 3: Mortality percentage of the *Ichthyophthirius multifiliis* theronts as a function of the concentration of the mixed extract (0.06-8 mL/l) and exposure time of 90 minutes. Formaldehyde (15 ppm) and aquarium water without any solution were considered positive and control treatments, respectively. Different statistical letters in each column indicate significant differences.

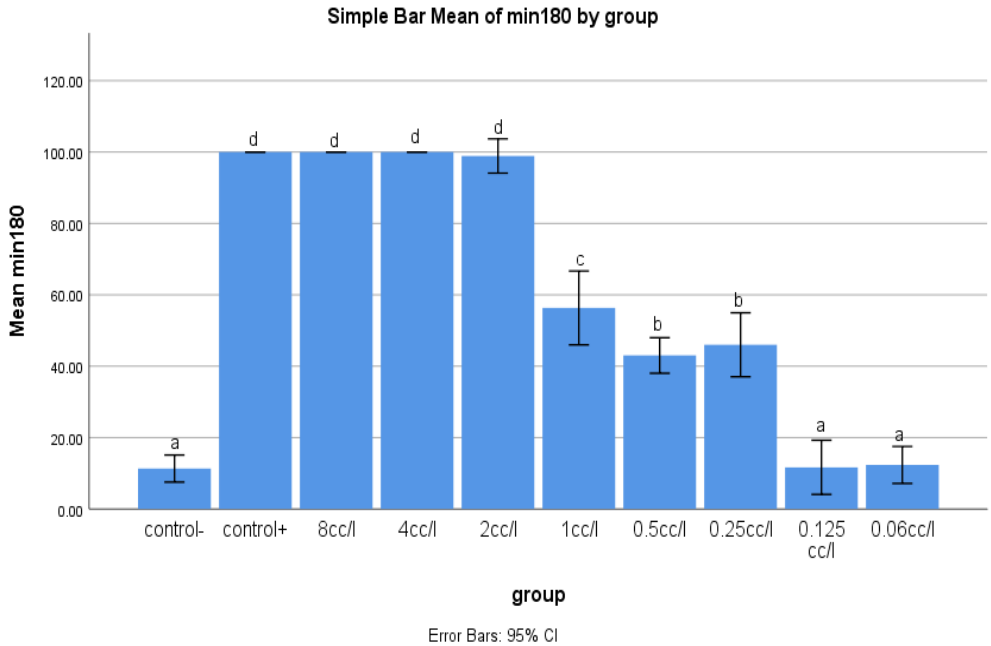

Figure 4: Mortality percentage of *Ichthyophthirius multifiliis* parasites as a function of mixed extract concentration (0.06-8 mL/l) and exposure time of 180 minutes. Formaldehyde (15 ppm) and aquarium water without any solution were considered positive and control treatments, respectively. Different statistical letters in each column indicate significant differences.

Discussion

Studies on the use of chemicals and extracts of medicinal plants to control some parasites, like *Ichthyophthirius multifiliis*, have gained more attention recently than in the past because of their great efficiency and low bio-environmental dangers. The

effects of two isolated flavonoids (kuwanons G and O) from a species of plant known by the scientific name *Morus alba* were examined by Liang *et al*. (2015). Their findings showed that these flavonoids had a 100% deadly effect on theronts. According to Yao *et al*. (2011), the application of dihydrosanguinarin, an alkaloid, at concentrations between 5.18 and 9.43 mg/liter has a very effective mortality coefficient on theronts in laboratory situation. Their earlier study (Yao *et al*., 2010) demonstrated that, in laboratory conditions, various sanguinarine doses taken from Macleaya cordata, a native plant in China, significantly killed parasite theronts.

Two alkaloid chemicals, chelerythrine and chloroxylonine, have been shown to have a severe fatal effect on parasite theronts in laboratory settings, according to Shan *et al*. (2014). Furthermore, Shinn *et al*. (2012) demonstrated that although theronts could not be completely eliminated by using a concentration of 1 mg/liter of bronopol chemical compound after 12 hours, theronts' infectivity was greatly decreased. Additionally, it has been noted that rainbow trout trophont populations decreased as a result of prolonged exposure to low bronopol concentrations (Picón-Camacho *et al*., 2012).

The effects of green velvet bean leaves and papaya seed crude methanol extract on the Ich parasite in goldfish (*Carassius auratus*) under in vitro and in vivo conditions were assessed in the study by Ekanem *et al*. (2004). After six hours, 100% of the parasites in vitro died at concentrations of 150 mg/liter of bean leaf extract and 200 mg/liter of papaya seed extract. Fish afflicted with parasites were immersed in baths containing green bean extract for 72 hours and papaya extract for 96 hours, both in an in vivo setting. Compared to the control group, there was a 90% reduction in the number of Ich parasites following treatment with the 200 mg concentration of both plant extracts.

Three active compounds-10-gingerol, 6-dehydroshogaol, and 6-dehydro-10-gingerol—were separated from the ginger plant by Fu *et al*. (2019) and their antiparasitic action against the Ich parasite in grass-eating carp was examined. In experimental settings, 10-gingerol exhibits the most antiparasitic activity of these three drugs. Thus, it completely destroys theronts, non-encapsulated tomonts, and encapsulated tomonts at concentrations of 2, 8, and 16 mg/liter, respectively. It achieves this by raising osmotic pressure and accumulating radicals. It damages membranes and releases free radicals. Due to its high tannic acid content, the tannin-rich tropical plant *Lysiloma latisiliquum* has been shown to have direct effects on the biology (i.e., size of parasitic worms and female fertility) of adult *Haemonchus contortus* when used for a brief period (Martinez-Ortiz-de-Montellano *et al*., 2010).

The antiparasitic efficacy of thirty plant species against the Ich parasite was studied (Yi *et al*., 2012). It was found that concentrations of 10 mg/L of the extracts of two plants, *Sophora alopecuroides* and *Magnolia officinalis*, may kill all Ich parasites after three and four hours in an in vitro condition. Our experiment's outcome and the study's outcome were identical. Additionally, Buchman *et al*.'s

investigation (Buchmann *et al*., 2003) into the impact of garlic extract on the Ich parasite revealed that, after 30 minutes, all of the parasites were destroyed at a dosage of 62.5 mg per liter. Our experiment's outcome and the study's outcome were identical.

Research has been done on the comparative in vitro and in vivo effects of feed additives, such as astaxanthin, coriander, oregano, thyme, and garlic, as an oral antiparasitic agent in rainbow trout. Garlic has the strongest killing power of these five herbs when applied to the parasite theront stage in an in vivo condition. Subsequently, astaxanthin, thyme, and oregano have demonstrated the greatest impact, although coriander has not demonstrated any anti-parasitic activity in lab condition. This study also measured plasma lysozyme activity to examine the immunological response, and the results indicate that these additions significantly raise plasma lysozyme activity. Therefore, by boosting the host's immune response, these additives both directly and indirectly stop the parasite from growing in the beginning (Mathiessen *et al*., 2021).

The results of an investigation into the effects of an ethanolic extract of Shirazi thyme (*Zataria multiflora*) on the tomont and theront stages of *Ichthyophthirius multifiliis* in zebrafish indicated that all the Ich theronts could be destroyed in 2.31 to 32.4 minutes with a concentration of 10 mL of the extract per liter and a time interval of 6.04 to 6.37 minutes for 20 mL of the extract. The findings of this study agreed with those of the present study. Additionally, using this extract greatly decreased the severity and prevalence of Ich infection as well as the growth and generation of the tomonts (Rahmati-Holasoo, *et al*., 2021).

Tannic acid's anti-parasitic properties have been investigated in vivo and in vitro on the ich theronts at concentrations of 0.75-7 mg/L for 1-3 hours (Alavinia *et al*., 2018; 2019; Rahmati TaghjehHassan *et al*., 2024). The mortality results from parasite theronts were comparable to the findings of this investigation. The number of parasite fatalities increased dramatically with the increase in tannic acid concentration from 0 to 7 mg/liter, and there was a direct and significant association between it and the compound's concentration. Additionally, there was a notable drop in parasite outbreaks when the exposure duration was extended from one to three hours (Alavinia *et al*., 2018; 2019).

The findings of Alijanpour *et al*. (2022) study on the effects of an alcoholic extract of *Chelidonium majus* L. on *Ichthyophthirius multifiliis* theronts under in vitro conditions suggested that there is a direct and significant correlation between the mortality rate of parasitic theronts and the extract's concentration and exposure time. Additionally, the mortality of the parasites increased significantly when the extract's concentration was increased from 0.1 to 6.4 gr/L. The most suitable and best-performing dosage was 6.4 with 100% lethality (Alijanpour *et al*., 2022).

The study of Rahmati-Holasoo *et al*. (2024), on the effects of the alcoholic Extract of Pomegranate Peel on *Ichthyophthirius multifiliis* Theronts, showed that concentrations of 2, 4, and 8 g/liter, in addition to 1 g/liter, which killed 96% of the theronts in 6 hours, could all

destroy the theronts. Furthermore, in this investigation, the fish completely perished at concentrations of 2, 4, and 8 g/L due to their extreme toxicity; nevertheless, concentrations of 1 and 0.5 g/L proved to be safe dosages after 96 hours. Furthermore, the EC50 value in this study was shown to be 1.41 g/liter. This research therefore demonstrated that the pomegranate peel alcoholic extract at 2, 4, and 8 g/l is only appropriate as an antiseptic and is not useful for clinical therapy. Thus, 1 g/L is advised for therapeutic treatment due to its excellent outcomes (Rahmati-Holasoo *et al*., 2024).

Conclusion

This study examined the impact of the present solution on the death rate of *Ichthyophthirius multifiliis* theronts at various times (45, 90, and 180 minutes) and concentrations (0, 06, 0.125, 0.25, 0.5, 1, 2, 4, and 8 ml/liter) under in vitro conditions. Different variables were used to determine the impact of parasiticides during the period, including the mortality rate and the proportion of parasitic survivorship. The amount of time needed to eradicate every parasite is another way to assess the potential of parasites. The study's findings demonstrated that, in vitro, *Ichthyophthirius multifiliis* is susceptible to the antiparasitic effects of mixture of thyme, mint, and ginger extract. The anti-parasitic qualities of this extract were found to significantly increase with increasing concentration and exposure duration. The concentrations of 8 and 4 mL/L of this extract had the strongest anti-parasitic impact, resulting in a 100% reduction in theronts in 45 minutes. Since the almost complete removal (95%) of parasitic theronts was also found at lower concentrations, it is suggested to use optimization methods to determine the ideal conditions for the complete removal of theronts. The general result indicated the high lethality of this disinfectant solution in destroying theronts. Of course, the investigation of supplementary tests of toxicity at lethal and sub-lethal levels in fish is a necessary condition and prerequisite for designing and making a reliable combination to control ichthyophthiriasis in the aquaculture industry.

References

Aguilar, A., Alvarez, M.F., Leiro, J.M. and Sanmartin, M.L., 2005. Parasite populations of the European eel (*Anguilla anguilla* L.) in the Rivers Ulla and Tea (Galicia, northwest Spain). *Aquaculture*, 249(**1-4**), 85-94. https://doi.org/10.1016/j.aquaculture.2005.04.052

Ahmadivand, S., Farahmand, H., Teimoori-Toolabi, L., Mirvaghefi, A., Eagderi, S., Geerinckx, T., Shokrpoor, S. and Rahmati-Holasoo, H., 2016. Boule gene expression underpins the meiotic arrest in spermatogenesis in male rainbow trout (*Oncorhynchus mykiss*) exposed to DEHP and butachlor. *General and Comparative Endocrinology*, 225, 235-241. https://doi.org/10.1016/j.ygcen.2015.05.011

Ahmadivand, S., Weidmann, M., El-Matbouli, M. and Rahmati-Holasoo, H., 2020. Low pathogenic strain of infectious pancreatic necrosis virus (IPNV) associated with recent outbreaks

in Iranian trout farms. *Pathogens*, 9(**10**), 782. https://doi.org/10.3390/pathogens9100782

Akbari, S., Mosavari, N., Tadayon, K. and Rahmati-Holasoo, H., 2014. Isolation of *Mycobacterium fortuitum* from fish tanks in Alborz, Iran. *Iranian Journal of Microbiology*, 6(**4**), 234.

Alavinia, S.J., Mirzargar, S.S., Rahmati-Holasoo, H. and Mousavi, H. E., 2018. The *in vitro* and in vivo effect of tannic acid on *Ichthyophthirius multifiliis* in zebrafish (*Danio rerio*) to treat ichthyophthiriasis. *Journal of Fish Diseases*, 41(**12**), 1793-1802. https://doi.org/10.1111/jfd.12886

Alavinia, S.J., Mirzargar, S.S., Rahmati-Holasoo, H. and Mousavi, H., 2019. *In vitro* investigation of short-term antiparasitic effect of tannic acid on *Ichthyophthirius multifiliis* theronts. *Journal of Veterinary Research*, 74(**2**), 219-227. https://doi.org/10.22059/JVR.2019.244686.2720

Alijanpour Z., Rahmati-Holasoo H., Ebrahimzadeh Mousavi H., Saeed Mirzargar S., Sharifzadeh A. and Nasiri A., 2022. In vitro study of effects of alcoholic extract of *Chelidonium majus* L. on *Ichthyophthirius multifiliis* theronts. *Journal of Fisheries*, 75(**3**), 405-417. https://doi.org/10.22059/JFISHERIES.2022.340970.1323

Buchmann, K., Sigh, J., Nielsen, C.V. and Dalgaard, M., 2001. Host responses against the fish parasitizing ciliate *Ichthyophthirius multifiliis*. *Veterinary Parasitology*, 100, 105-116. https://doi.org/10.1016/S0304-4017(01)00487-3

Buchmann, K., Jensen, P.B. and Kruse, K.D., 2003. Effects of sodium percarbonate and garlic extract on *Ichthyophthirius multifiliis* theronts and tomocysts: In vitro experiments. North *American Journal of Aquaculture*, 65(**1**), 21-24. https://doi.org/10.1577/1548-8454(2003)065<0021:EOSPAG>2.0.CO;2

Canella, M.F. and Rocchi-Canella, I., 1976. Biologie des Ophryoglenina (cilies hymenostermes, histophages). Annals of the University of Ferrara (N.S. Sect. 111), 3 (Suppl. 2), 1-510.

Corliss, J. 1979. The ciliated protozoa: Characterization, vlassification, and guide to the literature. Pergamon Press, Oxford, UK. pp: 455. https://doi.org/10.1016/C2013-0-02659-x

Dardona, Z., Amane, M., Dardona, A. and Boussaa, S., 2024. The anti-parasitic activity of *Thymus vulgaris* (Thyme): A literature review. *International Journal of Science and Research Archive*, 11(**1**), 2243-58. https://doi.org/10.30574/ijsra.2024.11.1.0302

Dickerson, H.W., 2006. *Ichthyophthirius multifiliis* and *Cryptocaryon irritans* (phylum Ciliophora). In: Woo, P.T.K. (ed.) Fish Diseases and Disorders. Volume 1. Protozoan and Metazoan Infections, 2nd edn. CABI Publishing, Wallingford, Oxon, UK. Pp 181-227.

Ekanem, A.P., Obiekezie, A., Kloas, W. and Knopf, K., 2004. Effects of crude extracts of *Mucuna pruriens* (Fabacea) and *Carica papaya* (Caricaceae) against

the protozoan fish parasite *Ichthyophthirius multifiliis. Parasitology Research*, 92(**5**), 361-366. https://doi.org/10.1007/s00436-003-1038-8

Finney, D.J., 1971. Statistical logic in the monitoring of reactions to therapeutic drugs. *Methods of Information in Medicine*, 10(**4**), 237-245. https://doi.org/10.1055/s-0038-1636052

Forouzan, S., Bahmani, M., Parsaei, P., Mohsenzadegan, A., Gholami-Ahangaran, M., Sadeghi, E., Saki, K. and Delirrad, M., 2012. Anti-parasitic activites of *Zingiber officinale* methanolic extract on *Limnatis nilotica*. *Glob Vet*, 9(2):144-8. https://doi.org/10.5829/idosi.gv.2012.9.2.63134

Forwood, J.M., Harris, J.O., Landos, M. and Deveney, M.R., 2015. Life cycle and settlement of an Australian isolate of *Ichthyophthirius multifiliis* Fouquet, 1876 from rainbow trout. *Folia Parasitologica*, 62, 013. https://doi.org/10.14411/fp.2015.013

Fu, Y.W., Zhang, Q., Xu, D.H., Xia, H., Cai, X.X., Wang, B., Liang, J., 2014a. Parasiticidal effects of Morus alba root bark extracts against *Ichthyophthirius multifiliis* infecting grass carp. *Diseases of Aquatic Organisms*, 108, 129-136. https://doi.org/10.3354/dao02708

Fu, Y.M., Zhang, Q.Z., Xu, D.H., Jiang, J.H. and Wang, B., 2014b. Antiparasitic effect of cynatratoside-C from *Cynanchum atratum* against *Ichthyophthirius multifiliis* on grass carp. *Journal of Agriculturral and Food Chemistry*, 62(**29**), 7183-7189. https://doi.org/10.1021/jf5018675

Fu, Y.W., Wang, B., Zhang, Q.Z., Xu, D.H., Liu, Y.M., Hou, T.L. and Guo, S.Q., 2019. Efficacy and antiparasitic mechanism of 10-gingerol isolated from ginger Zingiber officinale against *Ichthyophthirius multifiliis* in grass carp. *Veterinary Parasitology*, 265, 74-84. https://doi.org/10.1016/j.vetpar.2018.11.011

Gholipour-Kanani, H., Sahandi, J. and Taheri, A., 2012. Influence of garlic (*Allium sativum*) and mother worth (*Matricaria chamomilla*) extract on *Ichthyophtirius multifiliis* parasite treatment in sail fin molly (*Poecilia latipinna*) ornamental Fish. *APCBEE Procedia*, 4, 6-11. https://doi.org/10.1016/j.apcbee.2012.11.002

Hines, R.S. and Spira, D.T., 1973. *Ichthyophthirius multifiliis* (Fouquet) in the mirror carp, *Cyprinus carpio* L. I. Course of infection. *Journal of Fish Biology*, 5, 385-392. https://doi.org/10.1111/j.1095-8649.1973.tb04466.x

Larcombe, E., Alexander, M.E., Snellgrove, D., Henriquez, F.L. and Sloman, K.A., 2024. Current disease treatments for the ornamental pet fish trade and their associated problems. *Reviews in Aquaculture*. https://doi.org/10.1111/raq.12948

Liang, J.H., Fu, Y.W., Zhang, Q.Z., Xu, D.H., Wang, B. and Lin, D.J., 2015. Identification and effect of two flavonoids from root bark of *Morus alba* against *Ichthyophthirius multifiliis* in grass carp. *Journal of Agricultural and Food Chemistry*, 63(**5**), 1452-1459. https://doi.org/10.1021/jf505544e

Lin, T.L., Clark, T.G. and Dickerson, H., 1996. Passive immunization of channel catfish (*Ictalurus punctatus*) against the ciliated protozoan parasite *Ichthyophthirius multifiliis* by use of murine monoclonal antibodies. *Infection and Immunity*, *64*(**10**), 4085-4090. https://doi.org/10.1128/iai.64.10.4085-4090.1996

Ling, F., Wang, J.G., Lu, C., Wang, G.X., Lui, Y.H. and Gong, X.N., 2012. Effects of aqueous extracts of *Capsicum frutescens* (Solanaceae) against of the fish ectoparasite *Ichthyophthirius multifiliis*. *Parasitology Research*, 111, 841-848. https://doi.org/10.1007/s00436-012-2907-9

Ling, F., Lu, C., Tu, X., Yi, Y., Huang, A., Zhan, Q. and Wang, G., 2013. Antiprotozoal screening of traditional medicinal plants: Evaluation of crude extract of *Psoralea corylifolia* against *Ichthyophthirius multifiliis* in goldfish. *Parasitology Research*, 112, 2331-2340. https://doi.org/10.1007/s00436-013-3397-0

Maceda-Veiga, A., Salvadó, H., Vinyoles, D. and De Sostoa, A., 2009. Outbreaks of Ichthyophthirius multifiliis in redtail barbs *Barbus haasi* in a Mediterranean stream during drought. *Journal of Aquatic Animal Health*, 21(**3**), 189-194.https://doi.org/10.1577/H08-054.1

Malheiros, D.F., Maciel, P.O., Videira, M.N. and Tavares-Dias, M., 2016. Toxicity of the essential oil of *Mentha piperita* in *Arapaima gigas* (pirarucu) and antiparasitic effects on *Dawestrema* spp. (Monogenea). *Aquaculture*, 20, 455, 81-6. https://doi.org/10.1016/j.aquaculture.2016.01.018

Martínez-Ortíz-de-Montellano, C., Vargas-Magaña, J.J., Canul-Ku, H.L., Miranda-Soberanis, R., Capetillo-Leal, C., Sandoval-Castro, C.A., Hoste, H. and Torres-Acosta, J.F.J., 2010. Effect of a tropical tannin-rich plant *Lysiloma latisiliquum* on adult populations of *Haemonchus contortus* in sheep. *Veterinary Parasitology*, 172(**3-4**), 283-290. https://doi.org/10.1016/j.vetpar.2010.04.040

Mathiessen, H., Jaafar, R.M., Al-Jubury, A., von Gersdorff Jørgensen, L., Kania, P.W. and Buchmann, K., 2021. Comparative in vitro and in vivo effects of feed additives on rainbow trout response to *Ichthyophthirius multifiliis*. *North American Journal of Aquaculture*, 83(**2**), 67-77. https://doi.org/10.1002/naaq.10167

Matthews, R.A., 2005. *Ichthyophthirius multifiliis* Fouquet and ichthyophthiriosis in freshwater teleosts. *Advances in Parasitology*, 59, 159-241. https://doi.org/10.1016/S0065-308X(05)59003-1

Murray, A.G. and Peele E.J., 2005. A framework for understanding the potential for emerging diseases in aquaculture. *Preventive Veterinary Medicine*, 67, 223-235. https://doi.org/10.1016/j.prevetmed.2004.10.012

Picón-Camacho, S.M., Taylor, N.G., Bron, J.E., Guo, F.C. and Shinn, A.P., 2012. Effects of long duration, low dose bronopol exposure on the control of *Ichthyophthirius multifiliis* (Ciliophora),

parasitising rainbow trout (*Oncorhynchus mykiss* Walbaum). *Veterinary Parasitology*, 186(**3-4**), 237-244. https://doi.org/10.1016/j.vetpar.2011.11.022

Qu, S.Y., Liu, Y.H., Liu, J.T., Li, P.F., Liu, T.Q., Wang, G.X., Yu, Q. and Ling, F., 2024. Catechol compounds as dual-targeting agents for fish protection against *Ichthyophthirius multifiliis* infections. *Fish & Shellfish Immunology*, 151,109717. https://doi.org/10.1016/j.fsi.2024.109717

Rahmati-holasoo, H., Hobbenaghi, R., Tukmechi, A., Homayounimehr A. and Ghavami, R., 2010. Lymphocystis in a flower horn fish. *Comparative Clinical Pathology*, 19, 433–435. https://doi.org/10.1007/s00580-010-0993-6

Rahmati-Holasoo, H., Ahmadivand, S., Shokrpoor, S. and El-Matbouli, M., 2020. Detection of Carp pox virus (CyHV-1) from koi (*Cyprinus carpio* L.) in Iran; clinico-pathological and molecular characterization. *Molecular and Cellular Probes*. 54, 101668. https://doi.org/10.1016/j.mcp.2020.101668

Rahmati-Holasoo, H., Marandi, A., Mousavi, H.E. and Azizi, A., 2021a. Study of the losses of Siberian sturgeon (*Acipenser baerii*) due to gill infection with *Diclybothrium armatum* in sturgeon farms of Qom and Mazandaran provinces. *Animal Environment Journal*, 13(4), 193-200. https://doi.org/10.22034/aej.2021.165929

Rahmati-Holasoo, H., Moosavi, M.S.J., Mousavi, H.E., Mirzargar, S.S. and Mirghaed, A.T., 2021b. Effect of ethanol extract of *Zataria multiflora* on *Ichthyophthirius multifiliis* tomonts and theronts in *Danio rerio*. *Journal of Veterinary Research*, 76(**2**), 205-214. https://doi.org/10.22059/JVR.2019.283809.2946

Rahmati-Holasoo, H., Asadi, A., Nassiri, A., Ebrahimzadeh Mousavi, H. and Nabian, S., 2024a. In vitro Study of Effects of Alcoholic Extract of Pomegranate Peel on *Ichthyophthirius multifiliis* Theronts. *Journal of Medicinal Plants and By-Products*, 13, 644-652. https://doi.org/10.22034/JMPB.2024.366214.1713

Rahmati-Holasoo, H., Ebrahimzadeh Mousavi, H. A., Nassiri, A., Saeedi, D., Ghojaye-Yousefi, H., and Taheri Mirghaed, A. 2024b. Investigating contamination with external and internal parasites in ornamental freshwater angelfish (*Pterophyllum scalare*). *International Journal of Veterinary Research*, *4*(2), 41-53. https://doi.org/10.22034/4.2.41

Rahmati TaghjehHassan, J., Yakhchali, M., Nekoueifard, A., 2024. Study on effects of plants extracts of *Thymus vulgaris* and *Lavandula angustifolia* on *Ichthyophthirius multifiliis* in rainbow trout (*Oncorhynchus mykiss*). *Iranian Journal of animal Science*. https://doi.org/10.22059/IJAS.2024.367660.653970

Rintamäki-Kinnunen, P. and Valtonen, E.T., 1997. Epizootiology of protozoans in farmed salmonids at northern

latitudes. *International Journal for Parasitology*, 27(**1**), 89-99. https://doi.org/10.1016/S0020-7519(96)00162-2

Sadeghinezhad, J., Rahmati-holasoo, H., Fayyaz, S. and Zargar, A., 2015. Morphological study of the northern pike (*Esox lucius*) tongue. *Anatomical Science International*, 90, 235-239. https://doi.org/10.1007/s12565-014-0254-x

Sanabria, C., Diamant, A. and Zilberg, D., 2009. Effects of commonly used disinfectants and temperature on swim bladder non-inflation in freshwater angelfish, *Pterophyllum scalare* (Lichtenstein). *Aquaculture*, 293, (**3-4**), 158-165. https://doi.org/10.1016/j.aquaculture.2009.04.015

Sasani, F., Shokrpoor, S., Rahmati-Holasoo, H. and Zargar, A., 2016. Appearance of Red Mark Syndrome (RMS) in cultured rainbow trout (*Oncorhynchus mykiss* Walbaum, 1972) in Iran. *Bulletin of European Association of Fish Pathologists*, 36(**2**), 90-94.

Seyrafi, R., Najafi, G., Rahmati-Holasoo, H., Hajimohammadi, B. and Shamsadin, A.S., 2009. Histological study of hepatopancreas in iridescent shark catfish (*Pangasius hypophthalmus). Journal of Animal and Veterinary Advances*, 8(**7**), 1305-1307.

Sayrafi, R., Najafi, G., Rahmati-Holasoo, H. and Ghadam, M., 2011. Histological study of hepatopancreas in Hi Fin Pangasius (*Pangasius sanitwongsei*). *African Journal of Biotechnology*, 10(**17**), 3463. https://doi.org/10.5897/AJB10.1054

Shan, X.F., Kang, Y.H., Bian, Y., Gao, Y.h., Wang, W.L. and Qian, A.D., 2014. Isolation of active compounds from methanol extracts of *Toddalia asiatica* against *Ichthyophthirius multifiliis* in gold fish (*Carassius auratus*). *Veterinary Parasitology*, 199(**3-4**), 250-254. https://doi.org/10.1016/j.vetpar.2013.10.021

Shinn, A.P., Picón-Camacho, S.M., Bron, J.E., Conway, D., Yoon, G.H., Guo, F.C. and Taylor, N.G., 2012. The anti-protozoal activity of bronopol on the key life-stages of *Ichthyophthirius multifiliis* Fouquet, 1876 (Ciliophora). *Veterinary Parasitology*, 186(**3-4**), 229-236. https://doi.org/10.1016/j.vetpar.2011.11.025

Tieman, D.M. and Goodwin, A.E., 2001. Treatments for ich infestations in channel catfish evaluated under static and flow-through water conditions. *North American Journal of Aquaculture*, 63, 293-299. https://doi.org/10.1577/1548-8454(2001)063<0293:TFIIIC>2.0.CO;2

Van Den Bussche, R.A., Hoofer, S.R., Drew, C.P. and Ewing, M.S., 2000. Characterization of histone H3/H4 gene region and phylogenetic affinity of *Ichthyophthirius multifiliis* based on H4 DNA sequence variation. *Molecular Phylogenetics and Evolution*, 14(**3**), 461-468. https://doi.org/10.1006/mpev.1999.0700

Van, Q.P., Yılmaz, B.H. and Yavuzcan, H., 2021. *In vitro* antiparasitic activity of

ginger (*Zingiber officinale*) bulb and pomegranate (*Punica granatum*) peel against monogenean fish parasite., *Dactylogyrus* sp. *Acta Aquatica Turcica*, 27, 17(**1**), 56-63. https://doi.org/10.22392/actaquatr.751913

Wright, A.D. and Lynn, D.H., 1995. Phylogeny of the fish parasite *Ichthyophthirius* and its relatives *Ophryoglena* and *Tetrahymena* (Ciliophora, Hymenostomatia) inferred from 18S ribosomal RNA sequences. *Molecular Biology and Evolution*, 12(**2**), 285-290. https://doi.org/10.1093/oxfordjournals.molbev.a040203

Wurstbaugh, W.A. and Tapia, R.A., 1988. Mass mortality of fishes in Lake Titicaca (Peru–Bolivia) associated with the protozoan parasite *Ichthyophthirius multifiliis*. *Transactions of the American Fisheries Society*, 117(**2**), 213-217. https://doi.org/10.1577/1548-8659(1988)117<0213:MMOFIL>2.3.CO;2

Yao, J.Y., Shen, J.Y., Li, X.L., Xu, Y., Hao, G.J., Pan, X.Y., Wang, G.X. and Yin, W.L., 2010. Effect of Effect of sanguinarine from the leaves of *Macleaya cordata* against *Ichthyophthirius multifiliis* in grass carp (*Ctenopharyngodon idella*). *Parasitology Research*, 107, 1035-1042. https://doi.org/10.1007/s00436-010-1966-z

Yao, J.Y., Zhou, Z.M., Li, X.L., Yin, W.L., Ru, H.S., Pan, X.Y., Hao, G.J., Xu, Y. and Shen, J.Y., 2011. Antiparasitic efficacy of dihydrosanguinarine and dihydrochelerythrine from Macleaya microcarpa against *Ichthyophthirius multifiliis* in richadsin (*Squaliobarbus curriculus*). *Veterinary Parasitology*, 183, 8-13. https://doi.org/10.1016/j.vetpar.2011.07.021

Yazdani Anaraki E., Mirzargar S.S., Rahmati Holasoo H., Sharifzade A. and Ebrahimzade Musavi H.A., 2021. In vitro study of short-term antiparasitic effect of alcoholic extract of *Terminalia catappa* L. leaves on *Ichthyophthirius multifiliis* theronts. *Iranian Journal of Fisheries Sciences*, 20(**4**), 1138-1148. https://doi.org/10.22092/ijfs.2021.124565

Yi, Y.L., Lu, C., Hu, X.G., Ling, F. and Wang, G.X., 2012. Antiprotozoal activity of medicinal plants against *Ichthyophthirius multifiliis* in goldfish (*Carassius auratus*). *Parasitology Research*, 111, 1771-1778. https://doi.org/10.1007/s00436-012-3022-7

Zhang, Q.Z., Xu, D.H. and Klesius, P.H., 2013. Evaluation of an antiparasitic compound extracted from *Galla chinensis* against fish parasite *Ichthyophthirius multifiliis*. *Veterinary Parasitology*, 198(**1-2**), 45-53. https://doi.org/10.1016/j.vetpar.2013.08.019.