Parasiticidal effects of *Eclipta alba* and *Arctium lappa* extracts against *Ichthyophthirius multifiliis*

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**Abstract**

Ichthyophthiriasis, commonly known as white spot disease, occurs in both wild and cultured fish and is responsible for heavy economic losses to the aquaculture industry. In past decade, several chemical therapeutants were used to treat ichthyophthiriasis, but the effective drugs, such as malachite green, have been banned for use in food fish due to its genotoxic and carcinogenic properties. To find efficacious drugs to control *Ichthyophthirius multifiliis* (Ich), whole *Eclipta alba* plants and dried root of *Arctium lappa* were evaluated for their antiprotozoal activity. *E. alba* and *A. lappa* extracts significantly reduced the survival of Ich trophonts and theronts. *In vitro*, the *E. alba* and *A. lappa* methanol extracts killed all trophonts at 3200 mg l$^{-1}$. All trophonts were killed after exposure to *E. alba* aqueous extract at 3200 mg l$^{-1}$. The methanol extracts of *E. alba* and *A. lappa* killed 100% of *I. multifiliis* theronts at 400 mg l$^{-1}$ and 800 mg l$^{-1}$, respectively. The aqueous extract of *E. alba* and *A. lappa* killed 100% of *I. multifiliis* theronts at 1600 mg l$^{-1}$ and 3200 mg l$^{-1}$, respectively. *E. alba* and *A. lappa* extracts may be new and efficacious drugs for the control of ichthyophthiriasis.

**Key words:** fish, Ichthyophthirius multifiliis, parasite, Eclipta alba, Arctium lappa

**Introduction**

*Ichthyophthirius multifiliis* (Ich) is a ciliated protozoan parasitizing freshwater fish worldwide, which invades the gills and skin surfaces of fish. Ichthyophthiriasis, commonly known as white spot disease, occurs in both wild and cultured fish and is responsible for heavy economic losses to the aquaculture industry (Matthews 2005). The life cycle of *I. multifiliis* consists of an infective theront, a parasitic trophont, and a reproductive tomont (Zhang et al. 2013, Jørgensen 2017). The tomont stage includes nonencysted tomonts and encysted tomonts (Fu et al. 2014a).

In past decade, several chemical therapeutants were used to treat ichthyophthiriasis, such as formalin and sodium percarbonate (Forwood et al. 2014), copper sulfate (Ling et al. 1993), potassium permanganate (Straus and Griffin 2002), and malachite green. However, the effective drugs, such as malachite green, have been banned for use in food fish due to its genotoxic and...
carcinogenic properties (Srivastava et al. 2004). The most common treatments used in commercial aquaculture as alternatives to malachite green exhibit low efficacy, cause environmental problems, or are unlikely to receive regulatory approval (Dickerson and Dawe 1995, Tieman and Goodwin 2001). Therefore, it is necessary to find safe and effective antiparasitic agents to control ichthyophthiriasis.

_Eclipta alba_, also known as _Eclipta prostrata_ (family Asteraceae), grows commonly in moist areas as a weed all over the world. In many parts of India, it is grown commercially as a medicinal crop. _E. alba_ is widely used as a tonic agent and a diuretic and in the treatment of hepatic problems (Husain and Anis 2006). _E. alba_ extracts have been proven to contain coumestans, polypeptides, polyacetylenes, triterpenes, steroids, and flavonoids (Kumari et al. 2006). _E. alba_ has been described to have antiseptic, laxative, antibacterial, analgesic (Sawant et al. 2004), anthelmintic (Somnath et al. 2010), antiviral, antifungal (Sölleputra Boregowda et al. 2019), anti-inflammatory, and antihypertensive activity (Wong et al. 1988) and mosquito larvicidal and ovicidal properties (Govindarajan et al. 2011). Recently, researchers have reported that its extracts also possess anti-tumor properties (Saxena et al. 1993, Chaudhary et al. 2011).

Four compounds have been isolated from _E. alba_; two of them were identified as stigmasterol and alpha-terthienyl (Han et al. 1998, Song-Chow et al. 1998). Wedelolactone (coumestane), luteolin, and apigenin (flavonoids) are the three main bioactive polyphenolic constituents in _E. alba_ extracts (Manvar et al. 2012).

_Arctium lappa_, commonly known as burdock (family Asteraceae), is one of the most widely used plants in traditional Chinese medicine. _A. lappa_ root extracts contain several compounds, including flavonoids, lignans, tannins, phenolic acids, alkaloids, and terpenoids (Ferracane et al. 2010). Arctigenin (AR) and its glycoside, arctin, are two major active ingredients of _A. lappa_ (Gao et al. 2018). _A. lappa_ exhibits several biological activities (Chan et al. 2011), including antioxidant (Souza et al. 2018), antiinflammatory (Huang et al. 2010), anti-allergic (Knipping et al. 2008), antimicrobial (Holetz et al. 2002), antiulcer (Almeida et al. 2012), antidiabetic, hypolipidemic (Ahangarpour et al. 2017), and gastroprotective effects (Santos et al. 2008). In addition, it has been used in the treatment of hepatitis, gout, and many other inflammatory disorders (Chan et al. 2011, Nascimento et al. 2019). However, the anti-Ich activity of _A. lappa_ and _E. alba_ extracts has not been reported. The aim of this study was to investigate the parasiticidal effect of _A. lappa_ and _E. alba_ extracts against _I. multifilis_.

**Materials and Methods**

Whole _E. alba_ plants and dried root of _A. lappa_ were purchased from the herb wholesale company NANGA (Przemyslaw Figura, Złotów Poland) and ground with a stainless steel blender. The dry powder (20 g) of each species was extracted with 100 ml methanol and 100 ml of distilled water to obtain methanol and aqueous extracts, respectively. The powder samples were extracted at room temperature for 24 h. Each extract was collected by filtration using filter paper. This process was repeated 3 times for nearly complete extraction of all soluble constituents. The extract (pooled together all three batches of filtrates) was finally concentrated by evaporating the solvent with a rotary vacuum evaporator (IKO, Werke 05-ST) at 70°C and kept at 4°C until use. _E. alba_ and _A. lappa_ methanol extract concentrates were dissolved in 1 ml dimethyl sulfoxide (DMSO) and stored at -20°C until use.

The anti-Ich assay was performed according to the method described by Fu et al. (2014). _I. multifilis_ was isolated from the common carp (Cyprinus carpio), which was heavily infected with mature trophonts. In the anti-trophont experiment, approximately 40 trophonts in 100 μl of dechlorinated freshwater were placed into each well of a 96-well tissue culture plate. A solution of the methanol and aqueous extracts (100 μl) was added to each well in triplicate to make final concentration of 50, 100, 200, 400, 800, 1600, 3200, 6400, and 0 mg l⁻¹ (negative control), and formalin solutions at 50, 100, 200 mg l⁻¹ were used as positive controls. The final concentration of DMSO in the treatment was maintained at less than 0.25%.

Live and dead trophonts were identified based on their movement; the trophonts were considered dead if no motion of the parasite was observed. The trophonts were counted under a microscope (4x) 1, 2, 3, and 4 h post treatment. The presence of theronts was marked as positive (+) and their absence as negative (-). For the anti-theront experiment, the trophonts were transferred into glass beakers with 50 ml dechlorinated freshwater and incubated at 23°C for 18 h. After theronts were released, 100 μl of water containing approximately 200 theronts were placed into each well of 96-well microtitre plates. The theronts were exposed to the methanol and aqueous extracts at concentrations of 50, 100, 200, 400, 800, 1600, 3200, 6400, and 0 mg l⁻¹ (negative control), and to formalin solutions at 50, 100, and 200 mg l⁻¹ (positive control) in triplicate for each concentration. The status of the theronts (alive or dead) in each well was assessed 1, 2, 3, and 4 h post treatment. The presence of live theronts was marked as positive (+) and their absence as negative (-).
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Preparation of erythrocyte suspension

Five milliliters of blood was collected from the common carp in a tube containing heparin. The blood was centrifuged at 176 x g for three minutes in a laboratory centrifuge (Sigma, 3K30). Plasma (supernatant) was discarded and the pellet was washed three times with a sterile phosphate buffer saline solution (pH 7.2±0.2) by centrifugation at 176 x g for 5 min. The cells were resuspended in normal saline to 0.5%.

Hemolytic activity

The hemolytic activity assay was performed according to the method described by Kumar et al. (2011). In vitro hemolytic activity was assessed with a spectrophotometer. A volume of 0.5 ml of the cell suspension was mixed with 0.5 ml of the plant extracts (6400, 3200, and 1600 mg l⁻¹ concentrations in phosphate buffer saline). The mixtures were incubated for 30 min at 28°C in the incubator. Afterwards, the mixture was centrifuged at 176 x g for 10 min in a laboratory centrifuge. The free hemoglobin in the supernatant was measured in a UV-Vis spectrophotometer at 540 nm. Phosphate buffer saline and distilled water were used as minimal and maximal hemolytic controls. Each experiment was performed in triplicates at each concentration. The blood for testing was taken once, and then the fish were anesthetized with tricaine methane-sulfonate (MS-222) at a concentration of 200 mg l⁻¹ in a water bath. For this reason, the approval of the Ethics Committee was not required.

Results

This study has shown that the aqueous and methanol extracts of E. alba and A. lappa can kill I. multifiliis trophonts and therons. All trophonts were killed after 1 h of exposure to the E. alba methanol extract at the

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The values are means ± SD of 3 replicates.

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The values are means ± SD of 3 replicates.
concentration of 3200 mg l\(^{-1}\) and after 2 h of exposure to the \(A.\ lappa\) methanol extract at the same concentration (Table 1). Additionally, all trophonts were killed after 1 h of exposure to the \(E.\ alba\) aqueous extract at the concentration of 3200 mg l\(^{-1}\). The aqueous extract of \(A.\ lappa\) caused 35% mortality in the trophonts after 4 h of exposure at the concentration of 6400 mg l\(^{-1}\) (Table 2). The formalin solutions (positive control) killed 100% of \(I.\ multifiliis\) trophonts at the concentration of 100 mg l\(^{-1}\) within 1 hour. The \(E.\ alba\) methanol extract killed 100% of \(I.\ multifiliis\) trophonts at the concentration of 400 mg l\(^{-1}\) within 1 hour (Table 3). In turn, the \(A.\ lappa\) aqueous extract killed 100% of \(I.\ multifiliis\) trophonts at the concentration of 3200 mg l\(^{-1}\) within 4 hours (Table 4). The formalin solutions (positive control) killed 100% of \(I.\ multifiliis\) trophonts at the concentration of 100 mg l\(^{-1}\) within 1 hour.

In this study, the hemolytic activity of the aqueous and methanol extracts of \(E.\ alba\) and \(A.\ lappa\) was screened against normal fish erythrocytes. The results have demonstrated that, compared to formalin, the aqueous and methanol extracts from the whole \(E.\ alba\) plant and dried root of \(A.\ lappa\) are non toxic to fish erythrocytes. The highest concentration of \(E.\ alba\) and \(A.\ lappa\) extracts (6400 mg l\(^{-1}\)) did not damage fish erythrocytes.

### Discussion

Plants can be a source of new drugs because they contain countless natural products with a wide variety
of structures and pharmacological activities (Newman et al. 2003).

The results of previous studies evaluating plant extracts for their anti-Ich efficacy have suggested that crude extracts from some plants have compounds with significant effects against I. multifiliis and are potential resources for production of anti-Ich drugs (Buchmann et al. 2003, Ekanem et al. 2004, Ling et al. 2012, Yi et al. 2012, Fu et al. 2014, Puk and Guz 2021). On the other hand, crude plant extracts are usually less costly than purified plant compounds; hence, they may be used as efficacious and safe agents for controlling ichthyophthiriasis in aquaculture. Moreover, Chinese herbal medicines are natural and biodegradable and can reduce environmental risks (Valladao et al. 2015). The available literature provides no data on the efficiency of E. alba and A. lappa extracts against I. multifiliis. This paper is the first to evaluate the activity of E. alba and A. lappa methanol and aqueous extracts against I. multifiliis trophonts and theronts.

In conclusion, our results have demonstrated that the aqueous and methanol extracts from the whole E. alba plant and dried root of A. lappa can kill I. multifiliis trophonts and theronts. Additionally, they are non toxic to fish erythrocytes.

Further studies are needed to evaluate the effect of E. alba and A. lappa extracts to control ichthyophthiriasis in fish farms.

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References


