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Original article

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

P. Leśniak, K. Puk, L. Guz

Department of Biology and Fish Diseases, Faculty of Veterinary Medicine, University of Life Sciences, Akademicka 12, 20-033, Lublin, Poland

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. mulitifiliis* 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

Correspondence to: P. Leśniak, e-mail: paulina.lesniak@up.lublin.pl

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 (Sollepura 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-terthenyl (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, arctiin, 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. multifiliis*.

Materials and Methods

Whole *E. alba* plants and dried root of *A. lappa* were purchased from the herb wholesale company NANGA (Przemysław 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 (IKA, 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. multifiliis* 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 fresh water 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 microtiter 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 (-).

Table 1. Mortality of *Ichthyophthirius multifiliis* trophonts after being exposed to the methanol extracts of *Eclipta alba* and *Arctium lappa*

Concentration (mg l^{-1})	Mortality (%) of trophonts							
	Eclipta alba				*Arctium lappa*			
	1 h	2 h	3 h	4 h	1 h	2 h	3 h	4 h
0	0	0	0	0	0	0	0	0
50	0	0	10.6±2.1	27.3±2.3	0	0	0	30.7±2.1
100	0	24.7±3.8	48.3±3.2	63.0±3.0	0	20.3±2.5	28.7±1.5	59.7±2.0
200	35.5±10.0	65.2±1.5	70.7±3.5	80.3±2.1	0	43.6±3.1	65.2±1.5	83.3±3.5
400	70.7±3.5	72.0±3.0	83.3±3.5	90.7±2.5	35.5±10.0	70.7±3.5	83.3±3.5	90.0±1.1
800	80.3±2.1	92.0±2.6	94.3±2.1	98.3±2.8	67.3±2.1	79.3±2.1	94.3±2.1	98.3±2.8
1600	91.3±3.5	98.8±1.0	100±0.0	100±0.0	89.0±2.6	92.0±2.6	98.8±1.0	100±0.0
3200	100±0.0	100±0.0	100±0.0	100±0.0	94.0±1.8	100±5.5	100±0.0	100±0.0
6400	100±0.0	100±0.0	100±0.0	100±0.0	96.6±2.0	100±0.0	100±0.0	100±0.0

The values are means ± SD of 3 replicates.

Table 2. Mortality of *Ichthyophthirius multifiliis* trophonts after being exposed to the aqueous extracts of *Eclipta alba* and A*rctium lappa.*

Concentration (mg l^{-1})	Mortality (%) of trophonts							
	Eclipta alba				*Arctium lappa*			
	1 h	2 h	3 h	4 h	1 h	2 h	3 h	4 h
0	0	0	0	27.3±2.5	0	0	0	0
50	0	0	0	56.3±2.5	0	0	0	0
100	0	0	0	67.3±2.1	0	0	0	0
200	0	27.3±2.3	37.7±2.1	79.3±3.5	0	0	0	0
400	30.6±1.5	44.7±3.1	46.3±2.5	91.3±3.5	0	0	0	15.5±3.8
800	65.5±6.5	70.7±3.5	80.3±2.1	100±0.0	0	0	0	25.1±1.5
1600	90.3±2.5	92.0±4.6	98.3±2.1	100±0.0	0	0	0	30.0±1.8
3200	100±0.0	100±0.0	100±0.0	100±0.0	0	0	0	32.2±1.2
6400	100±0.0	100±0.0	100±0.0	100±0.0	0	0	0	35.4±1.5

The values are means ± SD of 3 replicates.

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

Table 3. Mortality of *Ichthyophthirius multifiliis* theronts after being exposed to the methanol extracts of *Eclipta alba* and *Arctium lappa*.

Concentration (mg l^{-1})	Mortality (%) of theronts							
	Eclipta alba				*Arctium lappa*			
	1 h	2 h	3 h	4 h	1 h	2 h	3 h	4 h
0	+	+	+	+	+	+	+	+
50	+	+	+	+	+	+	+	+
100	+	+	+	-	+	+	+	+
200	+	+	+	-	+	+	+	-
400	-	-	-	-	+	+	-	-
800	-	-	-	-	-	-	-	-
1600	-	-	-	-	-	-	-	-
3200	-	-	-	-	-	-	-	-
6400	-	-	-	-	-	-	-	-

The values are means ± SD of 3 replicates.
(+) Live theronts in the test wells, (-) No live theronts in the test wells

Table 4. Mortality of *Ichthyophthirius multifiliis* theronts after being exposed to the aqueous extracts of *Eclipta alba* and *Arctium lappa*.

Concentration (mg l^{-1})	Mortality (%) of theronts							
	Eclipta alba				*Arctium lappa*			
	1 h	2 h	3 h	4 h	1 h	2 h	3 h	4 h
0	+	+	+	+	+	+	+	+
50	+	+	+	+	+	+	+	+
100	+	+	+	+	+	+	+	+
200	+	+	+	-	+	+	+	+
400	+	+	+	-	+	+	+	+
800	+	-	-	-	+	+	+	+
1600	-	-	-	-	+	+	+	+
3200	-	-	-	-	+	+	+	-
6400	-	-	-	-	+	+	+	-

The values are means ± SD of 3 replicates.
(+) Live theronts in the test wells, (-) No live theronts in the test wells

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* theronts at the concentration of 400 mg l^{-1} within 1 hour (Table 3). Similarly, the *A. lappa* methanol extract killed 100% of *I. mulitifiliis* theronts at the concentration of 800 mg l^{-1} within 1 hour (Table 3). The aqueous extract of *E. alba* killed 100% of *I. multifiliis* theronts at the concentration of 1600 mg l^{-1} within 1 hour (Table 4). In turn, the *A. lappa* aqueous extract killed 100% of *I. mulitifiliis* theronts at the concentration of 3200 mg l^{-1} within 4 hours (Table 4). The formalin solutions (positive control) killed 100% of *I. multifiliis* theronts 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

Ahangarpour A, Heidari H, Oroojan AA, Mirzavandi F, Nasr Esfehani K, Dehghan Mohammadi Z (**2017**) Antidiabetic, hypolipidemic and hepatoprotective effects of *Arctium lappa* root›s hydro-alcoholic extract on nicotinamide-streptozotocin induced type 2 model of diabetes in male mice. Avicenna J Phytomed 7: 169-179.

Bhinge SD, Hogade MG, Chavan C, Kumbhar M, Chature V (**2010**) *In vitro* anthelmintic activity of herb extract of *Eclipta prostrate* L. against *Pheretima posthuma*. Asian J Pharm Clin Res 3: 229-230.

Boregowda RS, Murali N, Udayashankar AC, Niranjana SR, Lund OS, Prakash HS (**2019**) Antifungal activity of *Eclipta alba* metabolites against sorghum pathogens. Plants 8: 72.

Buchmann K, Jensen PB, Kruse KD (**2003**) Effects of sodium percarbonate and garlic extract on *Ichthyophthirius multifiliis* theronts and tomocysts: *in vitro* experiments. N Am J Aquac 65: 21-24.

Chan YS, Cheng LN, Wu JH, Chan E, Kwan YW, Lee SM, Leung GP, Yu PH, Chan SW (**2011**) A review of the pharmacological effects of *Arctium lappa* (burdock). Inflammopharmacology 19: 245-254.

Chaudhary H, Dhuna V, Singh J, Kamboj SS, Seshadri S (**2011**) Evaluation of hydro-alcoholic extract of *Eclipta alba* for its anticancer potential: an *in vitro* study. J Ethnopharmacol 136: 363-367.

De Almeida AB, Luiz-Ferreira A, Cola M, Di Pietro Magri L, Batista LM, de Paiva JA, Trigo JR, Souza-Brito AR (**2012**) Anti-ulcerogenic mechanisms of the sesquiterpene lactone onopordopicrin-enriched fraction from *Arctium lappa* L. (Asteraceae): role of somatostatin, gastrin, and endogenous sulfhydryls and nitric oxide. J Med Food 15: 378-383.

De Souza AR, Guedes AR, Rodriguez JM, Bombardelli MC, Corazza ML (**2018**) Extraction of *Arctium lappa* leaves using supercritical CO_2+ethanol: Kinetics, chemical composition, and bioactivity assessments. J Supercrit Fluids 140: 137-146.

Dickerson H, Dawe D (**1995**) *Ichthyophthirius multifiliis* and *Cryptocaryon irritans* (Phylum Ciliophora). In: Woo PTK (ed) Fish diseases and disorders, vol 1. Protozoan and metazoan infections. CAB International, Wallingford.

Dos Santos AC, Baggio CH, Freitas CS, Lepieszynski J, Mayer B, Twardowschy A, Missau FC, Santos ÉP, Pizzolatti MG, Marques MC (**2008**). Gastroprotective activity of the chloroform extract of the roots from *Arctium lappa* L. J Pharm Pharmacol 60: 795-801.

Ekanem AP, Obiekezie A, Kloas W, Knopf K (**2004**) Effects of crude extracts of *Mucuna pruriens* (Fabaceae) and *Carica papaya* (Caricaceae) against the protozoan fish parasite *Ichthyophthirius multifiliis*. Parasitol Res 92: 361-366.

Ferracane R, Graziani G, Gallo M, Fogliano V, Ritieni A (**2010**) Metabolic profile of the bioactive compounds of burdock (*Arctium lappa*) seeds, roots and leaves. J Pharm Biomed Anal 51: 399-404.

Forwood JM, Harris JO, Landos M, Deveney MR (**2014**) Evaluation of treatment methods using sodium percarbonate and formalin on Australian rainbow trout farms. Aquac Eng 63: 9-15.

Fu YW, Zhang QZ, Xu DH, Liang JH, Wang B (**2014a**) Antiparasitic Effect of cynatratoside-C from *Cynanchum atratum* against *Ichthyophthirius multifiliis* on Grass Carp. J Agric Food Chem 62: 7183-7189.

Fu YW, Zhang QZ, Xu DH, Xia H, Cai XX, Wang B, Liang J (**2014**) Parasiticidal effects of *Morus alba* root bark extracts against *Ichthyophthirius multifiliis* infecting grass carp. Dis Aquat Organ 108: 129-136.

Gao Q, Yang M, Zuo Z (**2018**) Overview of the anti-inflammatory effects, pharmacokinetic properties and clinical efficacies of arctigenin and arctiin from *Arctium lappa* L. Acta Pharmacol Sin 39: 787-801.

Govindarajan M, Karuppannan P (**2011**). Mosquito larvicidal and ovicidal properties of *Eclipta alba* (L.) Hassk (Asteraceae) against chikungunya vector, *Aedes aegypti* (Linn.) (Diptera: Culicidae). Asian Pac J Trop Med 4: 24-28.

Han Y, Xia C, Cheng X, Xiang R, Liu H, Yan Q, Xu D (**1998**) Preliminary studies on chemical constituents and pharmacological action of *Eclipta prostrata* L, Zhongguo Zhong Yao Za Zhi 23: 680-682.

Holetz FB, Pessini GL, Sanches NR, Cortez DA, Nakamura CV, Filho BP (**2002**) Screening of some plants used in the Brazilian folk medicine for the treatment of infectious diseases. Mem Inst Oswaldo Cruz 97: 1027-1031.

Huang TC, Tsai SS, Liu LF, Liu YL, Liu HJ, Chuang KP (**2010**) Effect of *Arctium lappa* L. in the dextran sulfate sodium colitis mouse model. World J Gastroenterol 16: 4193-4199.

Husain M, Anis M (**2006**) Rapid *in vitro* propagation of *Eclipta alba* (L.) Hassk. Through high frequency axillary shoot proliferation. Acta Physiol Plant 28: 325-330.

Jørgensen LG (**2017**) The fish parasite *Ichthyophthirius multifiliis* - Host immunology, vaccines and novel treatments. Fish Shellfish Immunol 67: 586-595.

Knipping K, van Esch EC, Wijering SC, van der Heide S, Dubois AE, Garssen J (**2008**) *In vitro* and *in vivo* anti-allergic effects of *Arctium lappa* L. Exp Biol Med (Maywood) 233: 1469-1477.

Kumar G, Karthik L, Rao KV (2011) Hemolytic activity of Indian medicinal plants towards human erythrocytes: An *in vitro* study. Elixir Appl Botany 40: 5534-5537.

Kumari CS, Govindasamy S, Sukumar E (**2006**) Lipid lowering activity of *Eclipta prostata* in experimental hyperlipidemia. J Ethnopharmacol 105: 332-335.

Lin SC, Yao CJ, Lin CC, Lin YH (**1998**) Hepatoprotective Activity of Taiwan Folk Medicine: *Eclipta prostrata* Linn. against various hepatotoxins induced acute hepatotoxicity. Phytother Res 10: 483-490.

Ling F, Wang JG, Lu C, Wang GX, Lui YH, Gong XN (**2012**) Effects of aqueous extract of *Capsicum frutescens* (Solanaceae) against the fish ectoparasite *Ichthyophthirius multifiliis*. Parasitol Res 111: 841-848.

Ling KH, Sin YM, Lam TJ (**1993**) Effect of copper sulphate on ichthyophthiriasis (white spot disease) in goldfish (*Carassius auratus*). Aquaculture (Netherlands) 118: 23-35.

Manvar D, Mishra M, Kumar S, Pandey VN (**2012**) Identification and evaluation of anti hepatitis C virus phytochemicals from *Eclipta alba*. J Ethnopharmacol 144: 545-554.

Matthews RA (**2005**) *Ichthyophthirius multifiliis* Fouquet and ichthyophthiriosis in freshwater teleosts. Adv Parasitol 59: 159-241.

Nascimento BA, Gardinassi LG, Silveira IM, Gallucci MG, Tomé MA, Oliveira JF, Moreira MR, Meirelles AF, Faccioli LH, Tefé-Silva C, Zoccal KF (**2019**) *Arctium lappa* extract suppresses inflammation and inhibits melanoma progression. Medicines (Basel) 6: 81.

Newman DJ, Cragg GM, Snader KM (**2003**) Natural products as sources of new drugs over the period 1981-2002. J Nat Prod 66: 1022-1037.

Puk K, Guz L (**2021**) Parasiticidal effects of *Tanacetum vulgare* extract against *Ichthyophthirius multifiliis*. Pol J Vet Sci 24: 159-161.

Sawant M, Isaac JC, Narayanan S (**2004**) Analgesic studies on total alkacoloids and alcohol extrats of *Eclipta alba* (Linn.) Hassk. Phytother Res 18: 111-113.

Saxena AK, Singh B, Anand KK (**1993**) Hepatoprotective effects of *Eclipta alba* on subcellular levels in rats. J Ethnopharmacol 40: 155-161.

Singh B, Saxena AK, Chandan BK, Agarwal SG, Anand KK (**2001**) *In vivo* hepatoprotective activity of active fraction from ethanolic extract of *Eclipta alba* leaves. Indian J Physiol Pharmacol 45: 435-441.

Srivastava S, Sinha R, Roy D (**2004**) Toxicological effects of malachite green. Aquat Toxicol 66: 319-329.

Straus DL, Griffin BR (**2002**) Efficacy of potassium permanganate in treating ichthyophthiriasis in channel catfish. J Aquat Anim Health 14: 145-148.

Tieman DM, Goodwin AE (**2001**) Treatments for Ich infestations in channel catfish evaluated under static and flow-through water conditions. N Am J Aquac 63: 293-299.

Valladao GM, Gallani SU, Pilarski F (**2015**) Phytotherapy as an alternative for treating fish disease. J Vet Pharmacol Ther 38: 417-428.

Wong SM, Antus S, Gottsegen A, Fessler B, Rao GS, Sonnenbichler J, Wagner H (**1988**) Wedelolactone and coumestan derivatives as new antihepatotoxic and antiphlogistic principles. Arzneimittelforschung 38: 661-665.

Yi YL, Lu C, Hu XG, Ling F, Wang GX (**2012**) Antiprotozoal activity of medicinal plants against *Ichthyophthirius multifiliis* in goldfish (*Carassius auratus*). Parasitol Res 111: 1771-1778.

Zhang Q, Xu DH, Klesius PH (**2013**) Evaluation of an anti-parasitic compound extracted from *Galla chinensis* against fish parasite *Ichthyophthirius multifiliis*. Vet Parasitol 198: 45-53.