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Research Article

DETERMINATION OF CONCENTRATION BOUNDARIES FOR THE TOXICITY OF *TERMINALIA CATAPPA LINN.* LEAVES EXTRACT ON HEALTHY *CARASSIUS AURATUS*

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ABSTRACT
Terminalia catappa from Family Combretaceae or locally known as *Ketapang* is naturally grows plant and abundantly available in many tropical areas. The medicinal properties such as anti parasitic and antibacterial of the leaves have been proven repeatedly in many scientific studies of aquaculture to treat infected fish. In the present study, toxicity level of different leaves extract concentrations on the healthy *Carassius auratus* or goldfish was investigated before the next study on its efficacy to treat the unhealthy *C. auratus* could be initiated. Ten *C. auratus* in 30-L tank aquarium were tested with five different concentrations of leaves extract; 400 mg/L, 500 mg/L, 600 mg/L, 800 mg/L and 1000 mg/L respectively and pH of bath extracts were determined before performing the tests. The results of pH are reduced or became acidic when the concentrations of leaves extract are increased significantly. During the test, toxicological signs were detected by physiological changes such as increased respiration frequency or erratic behavior. Overall results showed the methanolic extract of *T. catappa* leaves demonstrated a significantly high safety margin for the host of *C. auratus*. The *C. auratus* were well-tolerated with methanolic extract of *T. catappa* at the concentration of 400 mg/L for 3 hours without any visible effect. At 500 mg/L concentration, *C. auratus* also managed to survive for 3 hours but number of survived fish is reduced significantly different at ($P<0.05$) throughout 3 hours exposure time compared to control. Exposure to 600 mg/L, *C. auratus* were managed to survive up to 2 hours; meanwhile at 800 mg/L only up to 1 hour survived. At the highest concentration of 1000 mg/L, *C. auratus* have demonstrated an increased operculum movement and inconsistent behavior within few minutes of exposure time. However, no lethality of *C. auratus* was observed in the experimental period.

Keywords: *Carassius Auratus, Terminalia Catappa Leaves, Methanolic Extract, Toxicity Level*

INTRODUCTION
Malaysia has a reputation of exporting high quality freshwater ornamental fish. As importing countries have imposed regulations to prevent import of ornamental fish caught from the wild, in view of resulting depletion and extinction of natural stocks, demand for cultured freshwater ornamental fish is increasing (Andrews, 1990; Thilakaratne *et al.*, 2003). Consequently, the composition of export of fishery commodities in Malaysia specifically ornamental fish showed a raise of 24.59% totaling of 6,578 tonnes in 2011 compared to 5,280 tonnes in 2010 according to DOF, (2014). On top of that, Fisheries Statistic Report (Malaysia) on 2008 showed that among the popular groups of ornamental fish for their production are Cyprinids with 132.7 million from the total production of 733.2 million (DOF, 2014). One of the Cyprinid fish which is in demand and very popular throughout the world even today is *Carassius auratus* or goldfish.

Even this industry has tremendous potential for development; lack of understanding the disease situation is one that needed to be strengthened. Bondad-Reantaso *et al.*, (2005) has been reported an increase of disease outbreaks is due to culture intensification.

Physiological changes in fish such as stress or immunosuppression that heighten the susceptibility to infection are frequently because of overcrowding, periodic handling, high or sudden changes in temperature, poor water quality and poor nutritional status problems. Moreover, these physiological

changes will further facilitate the spread of pathogens and producing high mortality levels in fish (Cabello, 2006; Quesada *et al.*, 2013).

Hence, various chemical drugs and antibiotics have been used by most ornamental fish breeders to cope with the disease outbreaks and its deleterious consequences in aquaculture industry. In spite of that, the use of them is becoming more restricted since they present numerous side-effects for the environment and health safety (Reverter *et al.*, 2014). According to Intorre *et al.*, (2007), exposure to malachite green, formalin and potassium permanganate at the therapeutic dosage of parasitic diseases resulted in toxic effects, including death in Cyprinid fishes; goldfish and zebrafish. In other Cyprinid fish; carps have been shown to be suppressed during treatment with oxytetracycline and numbers of granulocytes were observed in the spleen of treated carp (Rijkers *et al.*, 1980). Considering of these potential harm, utilizations of the traditional plants in disease management are also increased for more economically, harmless, preventive and lasting methods. Numerous studies have been conducted with the extracts of various plants, screening for their antiparasitic, antibacterial and antifungal properties to replace chemical and antibiotic alternatives (Chitmanat *et al.*, 2005).

Terminalia catappa Linn. is a Combretaceous plant (tropical almond family), presenting throughout any region in Malaysia. In Southeast Asia, decoction of the leaves is used to treat indigestion and bronchitis; the crushed leaves mixed with coconut oil were used to relieve muscle pain from fractures and various extracts of the leaves and barks have been reported to possess anti-cancer, antioxidation, anti-inflammation and antifungal properties in modern pharmacological studies (Chansue and Assawawongkasem, 2008).

In aquaculture, the medicinal properties of the leaves have been proven repeatedly in scientific studies as antiparasitic and antibacterial properties.

Chitmanat *et al.*, (2005) reported that 0.8 mg/L concentration of its leaf extracts were proven effective against *Trichodina* and other bacterial and fungal infections in *tilapia*. In addition, the leaf extracts have also significantly decreased the number of *Gyrodactylus* and *Dactylogyrus* infections of *C. auratus* (Chansue and Tangtrongpiros, 2005).

Toxicity of plant extract towards the fish that reported in many laboratory studies may changes hematological, biochemical and physiological profiles of the fish and leading them to death ultimately (Omoniyi *et al.*, 2002; Tiwari and Singh, 2004; Ayotunde and Ofem, 2008). Besides of many successful treatments for infected fish resulted using *T. catappa* leaves extract, there is limited or no scientific data available regarding safety aspects of this remedy which can be used to ascertain the safety index of its preparation. An attempt has therefore been made under the present study to determine the toxicity level of *T. catappa* leaves extract against *C. auratus*; before the next investigation on its antiparasitic potential against infected *C. auratus* can be elucidated.

MATERIALS AND METHODS

Preparation of Plant Extract

The leaves of *T. catappa* were collected from its wildly grown plant; outside the Bestari Jaya Campus, University Selangor. Leaves were thoroughly washed with water to remove sands and debris. After that, the leaves were air-dried under low intensity sunlight at about 28^0C for 1 week and oven dried at 40^0C for another 48 hours. The dried leaves were then crushed and reduced to fine powder with a manual blender. Next, the powdered samples were freeze-dried at -45^0C to ensure complete removal of water. Subsequently, 100 g of *T. catappa* leaves dry powder were extracted with 1000 mL of methanol for about 48 hours according to the method of Yi *et al.*, (2012). Finally, the dried extracts were used for the preparation of the test solutions.

Preparation of Tested Fish

C. *auratus* with an average length of 4.0 - 6.0 cm and average weight of 4.5 $\pm$ 0.5 g were purchased from a local fish breeder in Selangor. All of them were acclimatized or maintained under laboratory conditions [temperature (20-22^0C), pH (6.8-7.5) and dissolved oxygen (6.0-7.8 mg/L)] for 7 days before test. They were fed once at 1% body weight daily with commercial fish pellet feed.

Toxicity Test

Toxicity of the *T. catappa L.* leaves extract at different concentrations of 400 mg/L, 500 mg/L, 600 mg/L, 800 mg/L and 1000 mg/L were tested on healthy *C. auratus* according to the method of Ekanem *et al.*, (2004); in each 30-L tank aquarium with two replicates. pH of the bath with different leaves extract concentrations were determined using a pH/redox meter before performing the test. All fishes were carefully observed for any sign of distress indicative of toxic insult. Under these circumstances, the fish were transferred immediately to fresh water.

Data Analysis

One-Way Analysis of Variance (ANOVA) is used for statistical analysis on the effect of methanolic extract of *T. catappa* leaves at different concentration on growth and survival of *C. auratus*. Significantly differences at ($P<0.05$) among means from different concentrations and control were distinguished using Tukey Test.

RESULTS AND DISCUSSION

The *C. auratus* with *T. catappa* methanolic bath extracts of 400 mg/L and 500 mg/L demonstrated similar survival rate with control for not more than 3 hours even there was significantly different at ($P<0.05$) in number of survived fish between both concentrations. Conversely, the *C. auratus* were well-tolerated and survived from 1 hour to 2 hours exposure time when the concentrations of leaves extract had increased from 600 mg/L up to 800 mg/L. Numbers of survived fish were also significantly different at ($P<0.05$) between both concentrations at 1 hour exposure time. In the highest concentration of bath extract at 1000 mg/L, all *C. auratus* had demonstrated early toxic effect between 2-5 minutes of bath exposure. Under these circumstances, all *C. auratus* were transferred to fresh water and returned to the normal condition within 24 hours. The full results of toxicity test were shown in Table 1.

Table 1: Number of *C. auratus* (n=10) Survived in the Different Bath Concentrations of *T. catappa* Leaves Extract within Exposure Time

Concentration of Leaves Extract (mg/L)	pH of Bath Extracts	Number of *C. Auratus* that Survived within Exposure Time		
		1 Hour	2 Hours	3 Hours
0 (Control)	6.8	$10.0^{b} \pm 0.0$	$10.0^{b} \pm 0.0$	$10.0^{b} \pm 0.0$
400	6.3	$10.0^{b} \pm 0.0$	$9.5^{b} \pm 0.71$	$9.5^{b} \pm 0.71$
500	6.2	$7.5^{b} \pm 0.71$	$1.0^{a} \pm 0.0$	$1.0^{a} \pm 0.0$
600	6.1	$6.5^{b} \pm 2.12$	$3.5^{a} \pm 2.12$	*
800	6.0	$1.5^{a} \pm 0.71$	*	*
1000	5.7	$0.0^{a} \pm 0.0$	*	*

Note:

*Experiment is not performed

Tests were performed with two replicates

Values with the different letter in the same column are significantly different ($P<0.05$) from control

The present study has showed the extracts are gradually acidic or the pH is decreased when their concentrations are increased significantly. Similarly, the numbers of survived fish were also reduced or decreased significantly comparing with control when the exposure time is increased. According to Helen and Brian, (2008) most aquarium fish live in water with a pH ranging from 5.5 to 8; where fish in freshwater aquariums generally do best with a neutral pH. Rapid fluctuations in pH are one of the toxicity problems towards the fish especially with low alkalinity pH of water; even most of them are usually well-tolerated with slow pH changes; not more than 0.3–0.5 units/d (Helen and Brian, 2008). There are many contributed factors for the pH decreased problem of water in the aquarium. A buildup of organic debris/organic acids is capable to decrease the pH and the rapid fluctuations can result in lethargy, stress, skin irritation/lesions, behavioral changes (such as attempting to jump out of the aquarium, flashing),

corneal edema, skin color changes, gill irritation with increased mucus production, respiratory signs and mortality (De la Torre *et al.*, 2000; Wildgoose, 2001; Barnhorn and Van Vuren, 2004; Osman *et al.*, 2010). Thus, later deteriorate effect can result in decreased immune system function and pre-disposing the fish to various infections of parasite, bacterial and viral. Chansue and Tangtrongpiros, (2005) also stated the acceptable pH for most fish cultures are ranged from 6.2 to 7.8 and it is has been proved that decrease of pH number over 0.2 may affect the fish growth and ultimately may cause mortality.

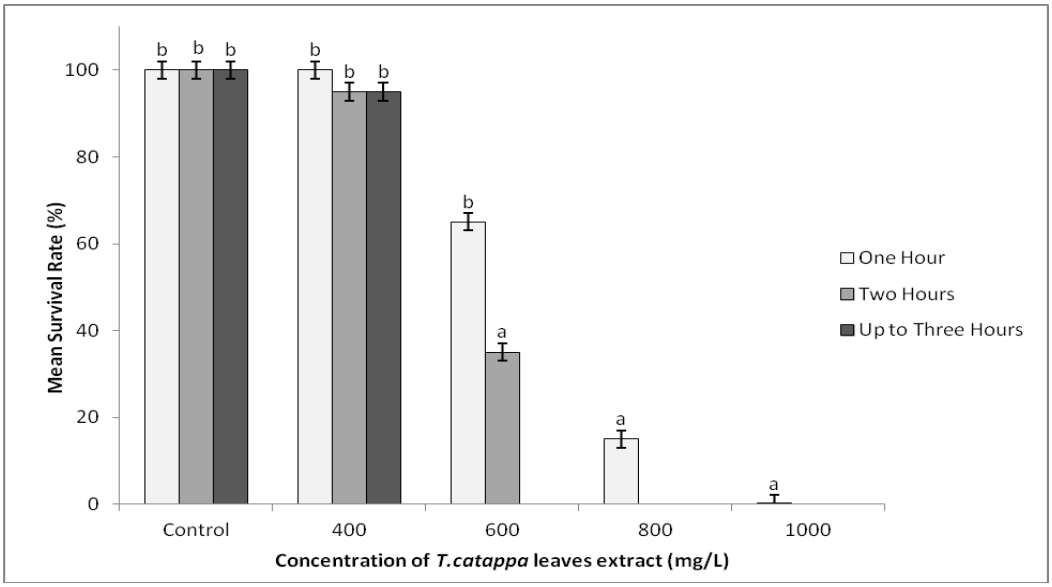

Figure 1: Survival Rate of *C. auratus* in Different Exposure Time and Concentrations of *T. catappa* Leaves Extract. Values with the Different Letter are Significantly Different (P<0.05) from Control

Condensed tannins derived from spruce bark that found in paper mills wastewater are not only toxic to methanogenic bacteria at particular concentrations; but they are also toxic to aquatic organisms due to the high reactivity of tannins with proteins (Field *et al.*, 1988). During the test observation, heavy solid suspensions from the *T. catappa* extracts were found and adhered at the gills of *C. auratus* in all tested concentrations; but adhered more in the concentrations of 800 mg/L and 1000 mg/L. These might be causes of toxic insult to the tested fish since *T. catappa* was found high content in organic materials, tannin and several flavonoids. Tannic acid from the extracts exhibits chelating properties when soaked in water and capable to form colloid that possibly causes adhesion in fish gills (Chansue and Tangtrongpiros, 2005). The adhered gills were blocked for the oxygen and the fish were ultimately irritated by high concentration of tannins. Since, the size of tested *C. auratus* is considerately small (4.0 - 6.0 cm length), it also can be another contributor for the low survival rate in the different bath concentrations of *T. catappa* leaves extract (Figure 1). In other toxicity study of tannins by Temmink *et al.*, (1989) severe gills damages and lesions had resulted in carp after being exposed to the concentration of tannins. These could be happened because of the osmo-regulatory functions of the carp gills are being disturbed and oligomeric of tannins had inactivated the membrane-bound transport ATP-ases in the mucosal epithelium theoretically (Temmink *et al.*, 1989; Segers *et al.*, 1984).

Conclusion

As a conclusion, the leaves extract of *T. catappa* had demonstrated a high safety margin for the host *C. auratus* since all of them had showed early toxic effects at 1000 mg/L concentration, after few minutes exposure time. Similarly, a study by Ekanem *et al.*, (2004) was also demonstrated an increased opercular movement and erratic behavior of the *C. auratus* within 30 minutes when exposure to 1000 mg/L leaves extract of *Mucuna pruriens*. Since, the concentration boundaries for *T. catappa* leaves extract toxicity were established, the study on its efficacy to treat infected *C. auratus* with parasite would be focused

next. Further phytochemical studies of *T. catappa* and toxicity tests to other fish species are also recommended.

REFERENCES

Andrews C (1990). The ornamental fish trade and conservation. *Journal of Fish Biology* [Online] **37** 53–59. Available: http://www.sciencedirect.com [Accessed 30 January 2016].

Ayotunde E and Ofem BO (2008). Acute and chronic toxicity of pawpaw (*Carica papaya*) seed powder to adult Nile tilapia (*Oreochromis niloticus Linna 1757*). *African Journal of Biotechnology* [Online] **7**(3) 22675–32274. Available: http://www.sciencedirect.com [Accessed 20 January 2016].

Barnhorn IEJ and Van Vuren JHJ (2004). The use of different enzymes in feral freshwater fish as a tool for the assessment of water pollution in South Africa. *Ecotoxicology and Environmental Safety* [Online] **59** 180–185. Available: http://www.sciencedirect.com [Accessed 20 January 2016].

Bondad-Reantaso MG, Subasinghe RP, Arthur JR, Ogawa K, Chinabut S, Adlard R, Tan Z and Shariff M (2005). Disease and health management in Asian aquaculture. *Veterinary Parasitology* [Online] **132** 249–272. Available: http://www.sciencedirect.com [Accessed 30 January 2016].

Cabello FC (2006). Heavy use of prophylactic antibiotics in aquaculture: a growing problem for human and animal health and for the environment. *Environmental Microbiology* [Online] **8** 1137–1144. Available: http://www.sciencedirect.com [Accessed 30 January 2016].

Chansue N and Tangtrongpiros J (2005). Effect of dried Indian almond leaf (*Terminalia catappa*) on monogenean parasite of goldfish (*Carassius auratus*) [Online]. In: *Proceeding of the 4th Chulalongkorn University Veterinary Annual Conference, Bangkok, Thailand*, 55-56. Available: http://www.sciencedirect.com [Accessed 5 April 2014].

Chitmanat C, Tongdonmuan K, Khanom P, Pachontis P and Nunsong W (2005). Antiparasitic, antibacterial and antifungal activities derived from a *Terminalia catappa* solution against some tilapia (*Oreochromis niloticus*) pathogens. *Acta Horticulturae* [Online] **678** 179-182. Available: http://www.sciencedirect.com [Accessed 5 April 2014].

De la Torre FR, Salibian A and Ferrari L (2000). Biomarkers assessment in juvenile *Cyprinus carpio* exposed to waterborne cadmium. *Environmental Pollution* [Online] **109** 277–282. Available: http://www.sciencedirect.com [Accessed 20 January 2016].

Department of Fisheries Malaysia (DOF). *Fisheries Statistic Report 2008* [Online] Malaysia Department of Fisheries. Available: http://www.dof.gov.my [Accessed 5 April 2014].

Ekanem AP, Obiekezie A, Kloas W and Knopf K (2004). Effects of crude of *Mucuna pruriens* (Fabaceae) and *Carica papaya* (Caricaceae) against the protozoan fish parasite *Ichthyophthirius multifiliis*. *Parasitology Research* [Online] **92** 361-366. Available: http://www.sciencedirect.com [Accessed 5 April 2014].

Field JA, Leyendeckers MJH, Sierra-Alvarez R, Lettinga G and Habets LHA (1988). The methanogenic toxicity of bark tannins and the anaerobic biodegradability of water soluble bark matter. *Water Science Technology* [Online] **20** 219-240. Available: http://www.sciencedirect.com [Accessed 2 February 2016].

Helen Roberts DVM, Brian S and Palmeiro VMD (2008). Toxicology of aquarium fish. *Veterinary Clinics Exotic Animal Practice* [Online] **11** 359-374. Available: http://www. sciencedirect.com [Accessed 2 February 2016].

Intorre L, Meucci V, Di Bello D, Monni G, Soldani G and Pretti C (2007). Tolerance of benzalkonium chloride, formalin, malachite green and potassium permanganate in goldfish and zebrafish. *American Veterinary Medical Association Journal* [Online] **231**(4) 590-595. Available: http://www.sciencedirect.com [Accessed 10 February 2014].

Omoniyi I, Agbon AO and Sodunke SA (2002). Effect of lethal and sublethal concentrations of tobacco (*Nicotiana tobaccum*) leaf dust extract on weight and hematologicalchanges in *Clarias gariepinus* (*Burch.*). *Journal of Applied Sciences and Environmental Management* [Online] **6**(2) 37–41. Available: http://www.sciencedirect.com [Accessed 20 January 2016].

Osman AGM, Koutb M and Sayed AEDH (2010). Use of hematological parameters to assess the efficiency of quince (*Cydonia oblonga Miller*) leaf extract in alleviation of the effect of ultraviolet—a radiation on African catfish *Clarias gariepinus* (Burchell,1822). *Journal of Photochemistry and Photobiology B:Biology* [Online] **99** 1–8. Available: http://www.sciencedirect.com [Accessed 20 January 2016].

Quesada SP, Paschoal JAR and Reyes FGR (2013). Considerations on the aquaculture development and on the use of veterinary drugs: special issue for fluoroquinolones—a review. *Journal of Food Science* [Online] **78** 1321–1333. Available: http://www.sciencedirect.com [Accessed 2 February 2016].

Reverter M, Bontemps N, Lecchini D, Banaigs B and Sasal P (2014). Use of plant extracts in fish aquaculture as an alternative to chemotherapy: Current status and future perspectives. *Aquaculture* [Online] **433** 50-61. Available: http://www.sciencedirect.com [Accessed 2 February 2016].

Rijkers GT, Teunissen AG, Van Oosterom R and Van Muiswinkel WB (1980). The immune system of cyprinid fish: the immunosuppressive effect of the antibiotic oxytetracycline in carp (*Cyprinus carpio L*). *Aquaculture* [Online] **19**(1) 77-89. Available: http://www.sciencedirect.com [Accessed 5 February 2016].

Segers JHL, Temmink JHM, Van-Den Berg JHJ and Wegman RCC (1984). Morphological changes in the gill of carp (*Cyprinus carpio L.*) exposed to acutely toxic concentrations of methylbromide. *Water Research* [Online] **18** 1437-1441. Available: http://www. sciencedirect.com [Accessed 2 February 2016].

Temmink JHM, Field JA, Van Hasstrecht JC and Merkelbach RCM (1989). Acute and sub-acute toxicity of bark tannins in carp (*Cyprinus carpio L.*). *Water Science Technology* [Online] **23**(3) 341-344. Available: http://www.sciencedirect.com [Accessed 2 February 2016].

Thilakaratne IDSIP, Rajapaksha G, Hewakopara A, Rajapakse RPVJ and Faizal ACM (2003). Parasitic infections in freshwater ornamental fish in Sri Lanka. *Diseases of Aquatic Organisms* [Online] **54** 157-162. Available: http://www.sciencedirect.com [Accessed 10 April 2014].

Tiwari S and Singh A (2004). Piscicidal and anti-acetyl cholinesterase activity of *Euphorbia royleana* stem bark extracts against freshwater common predatory fish *Channa punctatus*. *Environmental Toxicology and Pharmacology* [Online] **18** 47–53. Available: http://www.sciencedirect.com [Accessed 20 January 2016].

Wildgoose WH (2001). *BSAVA Manual of Ornamental Fish* [Online] 2nd edition, (Quedgeley, Gloucester (UK): British Small Animal Veterinary Association). Available: http://www.bsava.com/Publications/Buyonline/e-Books.aspx [Acessed 30 January 2016].

Yao JY, Shen JY, Li XL, Xu Y, Hao GJ, Pan XY, Wang GX and Yin WL (2010). Effect of sanguinarine from the leaves of *Macleaya cordata* against *Ichthyophthirius multifiliis* in grass carp (*Ctenopharyngodon idella*). *Parasitology Research* [Online] **107** 1035-1042. Available: http://www.sciencedirect.com [Accessed 4 April 2014].

Yi YL, Lu C, Hu XG, Ling F and Wang GX (2012). Antiprotozoal activity of medicinal plants against *Ichthyophthirius multifiliis* in goldfish (*Carassius auratus*). *Parasitology Research*, [Online] **111**(4) 1771-1778. Available: http://www.sciencedirect.com [Accessed 5 April 2014].