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ACUTE TOXICITY OF THE ETHANOL EXTRACT AND ETHYL ACETATE FRACTION OF *EUPATORIUM FORTUNEI* TO *DAPHNIA MAGNA*

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ABSTRACT

Previous studies have shown that the crude ethanol extract and its ethylacetate fraction of the plant *Eupatorium fortunei* Turcz strongly inhibited the growth of a harmful freshwater cyanobacterium *Microcystis aeruginosa*. However, the perspective of using these plant extracts as alternative algicides takes into account their potential risks to other species in aquatic ecosystems, including *Daphnia magna*. The current study presents the acute toxicity of the extracts to *D. magna*. The median lethal concentrations, immobilizing 50 % of *D. magna,* (LC_{50}) after 24 and 48 h of the ethanol extract were 247 and 183 mg L^{-1}, respectively. In the exposure to ethyl acetate fraction, the values of 24h-LC_{50} and 48h-LC_{50} were 47 and 13 mg L^{-1}, respectively. The values of dissolved oxygen (DO) and pH in the control and treatments had little change during the 48-hour experiment period, fluctuating from 6.44 ÷ 7.90 and 6.07 ÷ 7.78 mg L^{-1}, respectively, and they were still good conditions for *D.magna* growth. Finally, these results prove clearly that the ethyl acetate fraction is more toxic to the freshwater cladoceran than the ethanol extract. To our knowledge, it is the first report of the acute toxic estimation of *E. fortunei* extracts to zooplankton species, *D. magna*. Chronic toxicity of these extracts to *D. magna* needs to be studied in next step.

Keywords: Eupatorium fortunei, *Daphnia magna*, plant extracts, acute toxicity, LC_{50}.

1. INTRODUCTION

There has been various concerns on mass development of cyanobacteria in freshwater bodies due to their notorious impacts on aquatic ecosystems and human health through out the world [1]. Therefore, means of mitigation and control cyanobacterial bloom have been attracted by ecologists and environmentalists. Recently, methods to control cyanobacterial bloom and growth in fresh water ecosystem by using plant extracts have been investigated and introduced [2]. Among the cyanobacterial species, *Microcystis aeruginosa* is the most common species responsible for the water blooming and intoxication incidents. The previous investigations [3, 4] indicated that the plant extracts from *Cyperus rotundus, Chromolaena odorata, Callisia fragrans* and *Eupatorium fortunei* with their concentrations from 4 to 500 $\mu g.mL^{-1}$ effectively inhibited the growth of *M.aeruginosa*. Among the extracts, *Eupatorium fortunei* showed the highest anti-cyanobacteria properties at the concentration of 500 $\mu g.mL^{-1}$ with the inhibition efficiency (IE) of 95.5 % which were comparable with that of $CuSO_4$ at 5 $\mu g.mL^{-1}$ (IE of 81.7 %) [4]. Moreover, the extract was higher toxic to *M. aeruginosa* (IC_{50} of 119.3 $\mu g.mL^{-1}$) than to other green algae such as *Chlorella vulgaris* (IC_{50} of 315.1 $\mu g.mL^{-1}$).The ethyl acetate and water fractions from *E. fortunei* were also tested to combat the growth of the phytoplankton community and *Microcystis* population collected from the Hoan Kiem lake [5]. The authors reported that the significant inhibition of phytoplankton and *Microcystis*cell density was observed when the phytoplankton exposed to the ethyl acetate fraction at the concentration of 500 $\mu g\ mL^{-1}$ for 14 days. The IE value was 34.5 % for the *Microcystis* population, which was much higher than that for the phytoplankton (IE of 16.3 %). In term of the water fraction at 500 $\mu g\ mL^{-1}$, it showed the lower toxicity to both *Microcystis* species and phytoplankton with IE of 0.76 and 15.4 %, respectively. In another investigation, the toxicity of the extracts from *E. fortunei* to duckweeds (*Lemna minor* and *Spirodella polyrhiza)* was tested as representatives of sensitive non-target aquatic organisms to evaluate environmental safety [6].The significant growth inhibition of the extract on *M.aeruginosa* was reported at the 500 $\mu g\ mL^{-1}$ while *L.minor* was slightly affected by the extracts at the same concentration with IE of 25 % and *S.polyrhiza* was stimulated to about 5 % through fresh weight determinations. To provide a clear insight into the environmental safety of the *E. fortunei* extracts using as potential antialgal substances, their influenceson other non-target aquatic organisms need to be studied. Many species are used as the model organism for toxicity assessment such as green alga (*Ankistrodesmusconvolutus* and *Scenedesmusquadricauda),* duckweeds (*L. minor, S. polyrhiza),* freshwater cladoceran (*D. magna*), and phytoplanktons [6, 7, 8]. *D. magna* is the typical species in the toxicological studies and is used extensively in the studies evaluating the safety of plant extracts. This study aims to evaluate the acute toxicity of the ethanol and ethyl acetate extracts from *E. fortunei* to *D. magna.*

2. MATERIAL AND METHOD

2.1. *Daphnia magna* laboratory culture

Daphnia magna Strauss was purchased from MicroBioTests Inc., Belgium and used for the bioassay. This animal was raised in ISO medium containing $CaCl_2$, KCl, $NaHCO_3$ and $MgSO_4$ dissolved in reverse osmosis water (OECD, 1998). *Daphnia magna* was fed with a mixture of green algae *Chlorella* sp. and YTC (US.EPA, 2002). The organisms were kept at the temperature of 20 ± 2 °C and under a light density of around 1000 Lux and a photoperiod of 14 h light and 10 h dark.

2.2. Preparation of different extracts from *E. fortunei*

The aerial parts (leaves and stem) of *Eupatorium fortunei;* collected in January 2016 from Hoa Binh province and Soc Son district, Ha Noi, Viet Nam; were used for the experiment. The cleaned material was dried at room temperature to constant weight (5.19 kg), cut into small pieces and then ground into powder. Then, the powdered material was immersed separately in ethanol solvents 96 % (5L × 3 times) and subsequently macerated for two days at room temperature (23 ± 25 ^{0}C). The combined extracts were concentrated under vacuum to obtain the crude residue. This extract was resuspended in distilled water (2 L) and successively partitioned in hexane (1 L × 3 times) and ethyl acetate (1L × 3 times). Ethanol, ethyl acetate and hexane solvents were products of Merck (Germary). The ethyl acetate organic layers were concentrated to give ethyl acetate fraction, respectively. These extracts were kept at -5 ^{0}C for two weeks until use.

2.3. Acute toxicity test procedure and median lethal concentration calculation

Prior to conducting the experiment, fifty female *D. magna* were incubated in a beaker containing 500 mL ISO medium, and fed *ad libitum* with *Chlorella* sp. and YTC (OECD, 1998; US.EPA, 2002) over a period of two weeks in the laboratory conditions as mentioned above. The acute test was performed according to the US.EPA. (2002). Briefly, the *D. magna* neonates (< 24 h old) from the second or third brood were used for the acute test. The neonates were fed (green alga and YTC) ad libitum for 2 h before the test, but not during the exposure time. The neonates were exposed to either the ethanol crude extract at 7 different concentrations or the ethyl acetate fraction, from 0 to 400 µg ethanol extract mL^{-1} from 0 to 160 µg ethyl acetate fraction mL^{-1}. In each concentration, ten neonates were introduced into a flask containing 40 mL of ISO medium, and four replicates were prepared. The acute test was run in the dark and the death of animals was checked, and dead neonates were removed every 24 h or 48 h. The dead of neonates was confirmed by the stop of heart beat observed on a microscope [9]. The mortality data were utilized for determining median lethal concentration (LC_{50}) via EPA Probit Analysis Program.

3. RESULTS AND DISSCUSSION

3.1. Acute toxicity of the ethanol extract and ethyl acetate fraction from *E. fortunei* on *D.magna*

After 24 hours of ethanol extract's exposure, the mortality percentage of *D. magna* fluctuated from 0 % (for the control did not expose to the extract) to 85 % (for the sample adding the extract at 360 µg.mL^{-1}) and reached to 100 % (for the sample under the treatment of 400 µg.mL^{-1}). The mortality rate of *D.magna* was fastly increased after 48 hours exposure to the extract. In detail, the sample exposed the extract at 360 µg.mL^{-1}had already led to 100 % death of *D. magna.* Obviously from the Figures 1 and 2, the ethyl acetate fraction was greater toxic to *D.magna* than the ethanol extract. At the concentrations of 160 and 120 µg.mL^{-1} the ethyl acetate fraction killed all *D.magna* with mortality rate reached to 100 % after 24 and 48 hours, respectively. The 24h-LC_{50} and 48h-LC_{50} of the ethanol crude extract to *D.magna* were 247 and 183 µg.mL^{-1}, respectively. However, those of ethyl acetate fraction were just 47 and 13 µg.mL^{-1}, respectively (Table 1). To the best of our knowledge, no previous study was conducted to evaluate acute toxicity of *E. fortunei* extracts to *D. magna*.

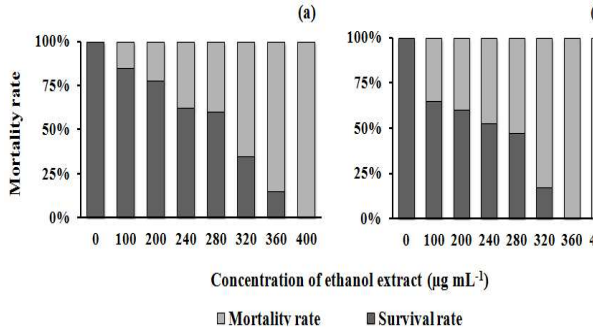

Figure 1. Acute toxicity of the ethanol extract from *E. fortunei* on *D. magna* after 24 (a) and 48(b) hours.

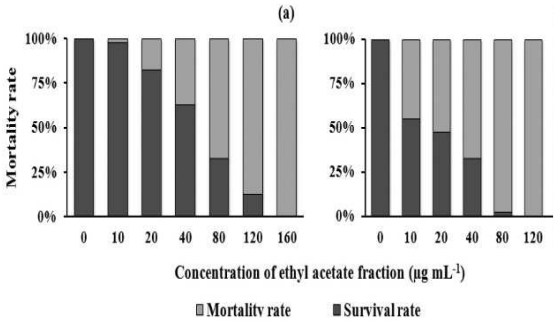

Figure 2. Acute toxicity of the ethyl acetate fraction from *E. fortunei* on *D. magna* after 24 (a) and 48 (b) hours.

Table 1. LC_{50} value of the crude ethanol extract and the ethyl acetate extract fraction after 24 and 48 hours.

Mortality Rate (%)	Concentration of the ethanol extract (µg.mL^{-1})		Concentration of the ethyl acetate fraction (µg.mL^{-1})	
	24 hours	48 hours	24 hours	48 hours
LC 1	71.4	37.0	7.8	1.8
LC 5	102.8	59.2	13.2	3.2
LC 10	125.0	76.0	17.6	4.4
LC 15	142.4	90.0	21.2	5.4
LC 50	**247.8**	**183.2**	**47.4**	**13.6**
LC 85	431.2	373.4	105.8	43.2
LC 90	491.6	442.0	128.0	42.4
LC 95	596.8	567.2	169.6	58.6
LC 99	859.2	885.8	287.8	107.4

However, Huang et al. [10] evaluated the acute toxicity of the different fractions obtained from methanol *E. fortunei* crude extracts on *Dactylogyrus intermedius* (Monogenea), a parasitic species of goldfish (*Carassius auratus*). The results showed that the chloroform fraction of *E. fortunei* had the most effective inhibition with an EC_{50} value of 85 mg.L^{-1}. The other fractions only caused weak effects on *D. intermedius (*EC_{50} from 100 to over 500 mg.L^{-1}). However, in comparison with the results of Park et al. [8], the ethanol extract from *Oryza sativa* showed the inhibition effect on the D.*magna* growth with IE of 53.3 % at the concentration of 1 mg.L^{-1} after 7 days of exposure. The other results about acute toxicity of plant extracts to *D.magna* were reported [7]. The aqueous extracts of five species of Family *Papaveracea* showed different inhibition effects on the growth of *D.magna* with their EC_{50} values ranged from over 32 to 1000 mg.L^{-1}.The aqueous extract from *Dicranostigma lactucoides* revealed the highest toxicity to *D. magna* (EC_{50} -24 h of 81 mg.L^{-1} and EC_{50} - 48h of 31 mg.L^{-1}) and following by that from *Sanguinaria canadensis* L. (EC_{50} 24h of 82 and EC_{50} 48h of 62 mg.L^{-1}). In our case, the ethanol extract from *E. fortunei* was less toxic to *D.magna* than the aqueous extracts from *D. lactucoides*

and S. canadensis. However, our ethyl acetate fraction indicated the most toxic to *D. magna* of all. The high total phenolic compounds of the ethyl acetate fraction could be responsible for the higher antibacterial and antifungal properties as well as more toxic to *D. magna* compared to the ethanol extract [11, 12].

3.2. Effect of the extracts on environmental variables DO and pH of the culture medium

There was no significant change in the DO and pH values during the 48 hours of experiment (Tables 2 and 3). The DO and pH of the samples exposed to ethanol crude extract at the concentrations of 0 ÷ 360 mg L^{-1} fluctuated from 6.83 to 7.92 mg L^{-1} and from 6.15 to 7.78, respectively, and those exposed to ethyl acetate fraction at the concentrations of 0 ÷ 160 mg.L^{-1} were 6.44 ÷ 7.88 mg.$L^{-1}$and 7.03 ÷ 7.77, respectively.

Table 2. DO and pH value of *D. magna* exposured to the ethanol extract from *E.fortunei* at 0 and after 48 hours.

Concentration of the ethanol extract (µgmL^{-1})	DO (T0)mg L^{-1}	DO (T48) mg L^{-1}	pH (T0)	pH (T48)
0.00	7.77	7.72	7.78	7.42
100.00	7.76	7.52	6.87	7.54
200.00	7.82	7.40	6.57	7.56
240.00	7.85	7.57	6.07	7.57
280.00	7.92	6.83	6.18	6.76
320.00	7.86	6.72	6.17	6.55
360.00	7.86	7.34	6.15	7.14

Table 3. DO and pH value of *D. magna* exposured to the ethyl acetate fraction from *E.fortunei* at 0 and after 48 hours.

Concentration of ethyl acetate fraction (µg mL^{-1})	DO (T0) mg L^{-1}	DO (T48) mg L^{-1}	pH (T0)	pH (T48)
0.00	7.77	7.42	7.77	7.42
10.00	7.87	7.51	7.78	7.49
20.00	7.85	7.44	7.70	7.44
40.00	7.88	6.88	7.65	7.37
80.00	7.83	6.44	7.52	7.17
120.00	7.86	6.92	7.44	7.15
160.00	7.85	7.72	7.29	7.03

They were still good conditions for *D. magna* growth. *D. magna* shows good survival, such as 85 % survival at the DO of 1.8 mg.$L^{-1}$and over 90 % at 2.7; 3.7 and 7.6 mg.L^{-1}. The Daphnia exposed to the lowest DO concentration tested (1.8 mg.L^{-1}) had significantly reduced responses for other parameters measured. In addition, the organisms exposed to 2.7 mg L^{-} O_2 gained less weight than did the controls [13]. In term of pH of the culture medium, it is important to aquatic life since it affects the normal physiological functions of aquatic organisms, including the exchange of ions with the water and respiration. According to the previous study, at the pH of 8.33, group of *D. magna* recorded the highest survival and growth rate and the optimum condition is from 7.9 to 8.3. When pH decreased from 4.66 to 4.44 and increased pH from 10.13 to 10.55 leading to the decrease in survival and growth rates of *Daphnia* species. In our study, the DO and pH values during the 48-hour experiment were in suitable range for *D. magna* growth [14]. Depending on the chemical composition of the plant, the pH of the sample-treated solutions could increase (flavonoid, alkaloid components) or decrease (in case of phenolic compounds). In our case, pH values of all treatments were slightly lower than those of the controls at the beginning and after 48 hours. It may be explained by the presence of similar compounds such as polyphenolic compounds in both ethanol extract and the ethyl acetate fraction. The difference in composition and ratio of flavonoids and phenolic acids in these extracts which led to the several chemical processes such as oxidation or reduction may occur which could be responsible for the changes in pH values [4, 11, 12].

4. CONCLUSION

The present work indicated that the ethanol extract and its ethyl acetate fraction of *Eupatorium fortunei* Turcz had the different acute toxicity to *D. magna* after 24 and 48 hours of exposure. The ethanol extract was less toxic to *D. magna* with LC_{50}-24h and LC_{50}-48h of 247 and 183 mg.L^{-1}, respectively. In terms of ethyl acetate fraction, the values of LC_{50}- 24h and LC_{50}-48h were 47 and 13 mg.L^{-1}, respectively. The values of dissolved oxygen (DO) and pH in the control and treatments measured during the 48-hour experiment period were still in the suitable ranges for *D.magna* growth.

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REFERENCES

1. Chorus I. and Bartram J. - A guide to their public health consequences, monitoring and management, Toxic Cyanobacteria in Water –. E & FN Spon, London. 1999, ISBN 0-419-23930-8.
2. Shao J., Renhui L., Joe E. L., Ji-Dong G. - Potential for control of harmful cyanobacterial blooms using biologically derived substances problems and prospects, Journal of Environmental Management **125** (2013) 149- 155.
3. Nguyen T. D., Duong T. T., Le T. P. Q., Ho T. C., Vu T. N., Pham T. Nga, Dang D. K. - Study of antibacterial properties of several plant extracts to *Microcystis aeruginosa*, Journal of Chemistry **51** (2C) (2013) 737-739 (in Vietnamese).
4. Pham T. N., Pham H. D., Nguyen. V. Q., Tran H. T., Le T. P. Q., Nguyen T. D., Duong T. T., Dang D. K. - Inhibitory effect of different *Eupatorium fortunei* Turcz extracts on the

growth of *Microcystis aeruginosa*, Vietnam Journal of Science and Technology **55** (4B) (2017) 103-108.

5. Duong T. T., Ho T. C., Le T. P. Q., Nguyen T. D., Pham T. N., Vu T. N., Dang D. K. - Growth inhibition of phytoplankton communities collected from Hoan Kiem Lake by different solvent extracts from *Eupatorium fortune* TURCZ, Journal of Biology **37**(2) *(*2015*)* 164-169 (in Vietnamese).
6. Pham T. N., Tran T. B., Pham H. D., Nguyen V. Q., Le T. P. Q., Nguyen T. D., Duong T. T., Dang D. K. - Influence of *Eupatorium fortunei* Turcz extracts on the growth of *Lemna minor* and *Spirodela polyrhiza*. Proceeding of The 5th Academic Conference on Natural Science for Young Scientists, Master and PhD. Students from Asean Countries (2017) 104-111, ISBN: 978-604-913-088-5.
7. Jancula D., Suchomelová J., Gregor J., Smutná M., Marsálek B., Táborská E. - Effects of aqueous extracts from five species of the family *Papaveraceae* on selected aquatic organisms, Environmental Toxicology **2** (5) (2007) 480-6.
8. Park MH., Kim BH., Chung IM., Hwang SJ. - Selective bactericidal potential of rice (*Oryza sativa* L. var. *japonica*) hull extract on Microcystis strains in comparison with green algae and zooplankton, Bulletin of Environmental Contamination Toxicology **83** (1) (2009) 97-101.
9. Dao T. S., Do-Hong L. C., Wiegand C. - Chronic effects of cyanobacterial toxins on *Daphnia magna* and their offspring. Toxicology **15**; 55(7) 2010 1244-54.
10. Huang A.G., Yi Y. L., Ling F., Lu L., Zhang Q. Z., Wang G. X. - Screening of plant extracts for anthelmintic activity against *Dactylogyrusintermedius* (Monogenea) in goldfish (*Carassiusauratus*), Parasitology Research **112** (2013) 4065–4072.
11. Nakamura M., Ra J., Jee Y., Kim J. - Impact of different partitioned solvents on chemical composition and bioavailability of *Sasa quelpaertensis* Nakai leaf extract, Journal of food and drug analysis **25** (2017) 316- 326.
12. Do Q. D., Angkawijaya A. E., Tran-Nguyen P. L., Huynh L. H., Soetaredjo F. E., Ismadji S., Ju Y. H. – Effect of extraction solvent on total phenol content, total flavonoids content, and antioxidant activity of *Limnophilaaromatica*, Journal of Food and Drug Analysis **22** (3) (2014) 296-302.
13. Homer D. H., William T. W. - Chronic effects of reduced dissolved oxygen on *Daphnia magna*, Water Air and Soil Pollution **20** (1) (1983) 23-28.
14. Mahassen M., Ghazy E-Deeb., Madlen M. H., Eman Y. M. - Effects of pH on Survival, Growth and Reproduction Rates of The *Crustacean*, *Daphnia magna*. Australian Journal of Basic and Applied Sciences **5** (11) (2011) 1-10, ISSN 1991-8178.