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Inhibitory Effects of *Ludwigia Octovalvis* (Jacq.) Raven Extracts on the Growth of *Microcystis Aeruginosa*

Nguyen Xuan Hoa, Tran Thi Thuy, Vu Thi Huyen, Phung Thi Vinh, Pham Trung Duc & Doan Thi Thuy Ai*

Faculty of Natural Resources and Environment, Vietnam National University of Agriculture, Hanoi 131000, Vietnam

Abstract

This study examined the phytochemical composition and algicidal effectiveness of *Ludwigia octovalvis.* The powder samples were extracted by ultrasound-assisted extraction with polar solvents (water-diluted ethanol, acetone, methanol, and water). The preliminary phytochemical analyses used standard procedures following Sofowora and Harborne. Total phenolic contents in extracts were determined by the Folin-Ciocalteu method using a calibration curve of gallic acid. The results showed that this plant contains polyphenols, flavonoids, anthraquinones, glycosides, and saponins. The best conditions for the extraction of polyphenol compounds with a total polyphenol content of 149.22 ± 0.96 mg GAE g^{-1} were acetone/water 70:30 (v/v) and a solvent-to-material ratio of 20 mL g^{-1}. The inhibitory effect of the extracts against *M. aeruginosa* growth increased from 40.71 to 81.56% on day 7 when exposed to concentrations of the extract from 50-200 μg mL^{-1} according to the cell counting method. The *L. octovalvis* extract was identified as an effective inhibitor of the growth of *M. aeruginosa.*

Keywords

Algae, cyanobacteria, *Microcystis aeruginosa*, *Ludwigia octovalvis*, Onagraceae

Introduction

Microcystis aeruginosa is one of the most common types of toxic cyanobacteria. It produces cyclic peptide compounds called microcystins (Ding *et al.*, 1998; Oh *et al.*, 2000), which cause many serious diseases in animals and humans (Nishiwaki-Matsushima *et al.*, 1992; Rao *et al.*, 1995). Therefore, controlling *M. aeruginosa* growth is one of the most important solutions to deal with environmental pollution caused by toxic algae.

Previous research has documented the use of substances such as copper sulfate (Han *et al.*, 2001), potassium permanganate,

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Correspondence to
dttai@vnua.edu.vn

hydroperoxide (Jančula & Maršálek, 2011; Fan *et al.*, 2014), and nano metals or metal oxides as algicidal agents (Karan *et al.*, 1998; Sankar *et al.*, 2014). These chemical algicides can remove toxic algae easily and quickly. However, due to their non-selective characteristics, they can be harmful to fish and other aquatic animals, so they also cause secondary pollution and lead to the deterioration of water quality.

Utilizing allelopathic plant compounds as substitutes for traditional algicidal drugs has emerged as a highly intriguing strategy in recent years to manage cyanobacteria outbreaks. Numerous researchers have examined the ability of plant extracts and bioactive substances to inhibit the growth of *M. aeruginosa* (Barrett *et al.*, 1999; Shao *et al.*, 2013; Meng *et al.*, 2015; Nga *et al.*, 2017). Polyphenols are organic substances that have a variety of fascinating biological functions, including having antioxidant, antiviral, and antibacterial properties. These compounds have been identified as the primary bioactive components in herbal extracts that prevent cyanobacteria from growing (Pillinger *et al.*, 1994; Nakai *et al.*, 2001; Huang *et al.*, 2015; Tebaa *et al.*, 2017). By bioassay-guided fractionation, eugeniin and its derivatives, ellagic and gallic acids, were obtained from *Myriophyllum spicatum*, and all of these polyphenols showed significant inhibitory activity in the growth of *M. aeruginosa* (Gross *et al.*, 1996). *M. aeruginosa* growth was also inhibited by p coumaric acid and vanillic acid, with EC_{50} values of 0.26 ± 0.07 and 0.34 ± 0.05 mmol L^{-1}, respectively (Zhang *et al.*, 2010).

Ludwigia octovalvis (Jacq.) Raven belongs to the family Onagraceae. The tree is an undershrub, erect, up to 2.5m high, well-branched, and widely distributed in America, Africa, Asia, and Australia (Chen *et al.*, 2007). The plant is a traditional herbal remedy in Vietnam to treat gastrointestinal conditions like flatulence and diarrhea.

Several *Ludwigia* species have been studied to learn more about their chemical compositions and bioactivities, including *L. octovalvis, L. hyssopifolia, L. adscendes, L. leptocarpa,* and *L. peploides*. Previous studies have shown that *Ludwigia* species are diverse in polyphenol compounds and have many bioactivities such as having antioxidant, antimicrobial, and anticancer properties (Wu *et al.*, 2010; Yakob *et al.*, 2012; Yakob *et al.*, 2015; Smida *et al*., 2018; Baky *et al*., 2022; Shawky *et al*., 2023). However, the plants have not yet been studied in terms of their ability to inhibit the growth of cyanobacteria.

In this study, we concentrated on the identification of the phytochemicals present in *L. octovalvis, the* preparation of a rich polyphenol extract from *L. octovalvis,* and the evaluation of the growth-inhibitory effects of the extract on *M. aeruginosa* bacteria.

Materials and Methodology

Materials

The cyanobacterium *M. aeruginosa* was collected from four lakes at Vietnam National University of Agriculture (VNUA) in 2019 and cultured in nutrient B12-medium, with approximately 40 µmol photons m^{-2} s^{-1} provided by cool white fluorescent lamps, a temperature of 28^{o}C, and a light cycle of (12 light: 12 dark) following the previously reported methods of Nakagawa *et al.* (1987).

The aerial parts of *L. octovalvis* were collected in February 2019 from Hung Yen province, Vietnam, air-dried in the shade, and ground to a powder. The plant was identified by Dr. Do Van Truong, Department of Biology, Vietnam Academy of Science and Technology. A voucher specimen (VTH-01) was deposited at the Department of Biology, Vietnam National Museum of Nature.

Chemicals

Folin-Ciocalteu reagent and gallic acid were purchased from Sigma-Aldrich (USA). $NaNO_3$, K_2HPO_4, $MgSO_4$-$7H_2O$, $CaCl_2$-$2H_2O$, ferric citrate, Na_2EDTA, Na_2CO_3, $FeCl_3$, vitamin B12 (China) to form the nutrient B12-medium, solvents, and other chemicals were of analytical grade.

(a) (b) (c)

Figure 1. The materials

a, b/ Twigs of L.octovalvis with flowers; c/ *M.*aeruginosa

Methods

Phytochemical screening

The phytochemical tests were performed on the *L. octovalvis* extracts using standard methods (Sofowora, 1996; Harborne, 1998). Secondary groups, namely phenolics, flavonoids, anthraquinones, glycosides, and saponins, were identified in this work.

(i) Test for phenolics

Two milliliters (2mL) of iron (III) chloride 1% was added to 2mL of *L. octovalvis* extract. A bluish-green or green color showed the presence of phenols.

(ii) Test for flavonoids

Flavonoids were indicated by the alkaline test. A few drops of 2M sodium hydroxide solution was added to 2mL of extract. The presence of flavonoids changed the color of the solution to yellow or red.

(iii) Test for anthraquinones

A mixture of extract and H_2SO_4 3M was shaken and extracted with chloroform. The chloroform layer was diluted with ammonia 10%. A pink aqueous ammonia layer indicated the presence of anthraquinones.

(iv) Test for glycosides

Two milliliters (2mL) of acetic acid and 2mL of chloroform were added to 2mL of the extract. The solution was cooled and concentrated H_2SO_4 was added. The presence of glycosides made the solution turn green.

(v) Test for saponins

The foam test was used to indicate the presence of saponins. A mixture of 2mL of distilled water and 2mL of the extract was shaken vigorously. The presence of saponin compounds made foam appear on the surface for ten minutes.

Extraction of polyphenols

A sample of *L. octovalvis* (0.2g) was extracted three times by ultrasound-assisted extraction (20kHz, 400W) for 20min at 30°C. The extract solution was concentrated using a rotary evaporator (I-300, Buchi, Switzerland) at 40°C to obtain the extract. The extract was stored at 4°C until further analysis.

In order to study the conditions of the extraction process various factors were changed in single-factor experiments, namely the extraction solvent types, the concentrations of the aqueous solvent, and the solvent-to-material ratios.

Determination of the total phenolic content

The value of total polyphenol (TP) content was determined by using the Folin-Ciocalteu method (Singleton & Rossi, 1965). A mixture of 2.5mL of Folin-Ciocalteau reagent (1/10 diluted)

and 0.5mL of the plant extract solution was shaken and incubated at room temperature (28°C) for 5min. Then, 2mL of sodium carbonate 7.5% was added to the mixture and incubated again in the dark for 2 hours. The absorbance of the mixture was measured at 760nm using a DR3900 UV-VIS spectrophotometer (Singleton & Rossi, 1965). The TP was expressed as micrograms of gallic acid equivalents per gram of dry sample (mg GAE g^{-1} using the standard curve of gallic acid ($y = 0.0087x + 0.0119$, $R^2 = 0.9991$).

Algal bioassay

The inhibitory action on algal growth was evaluated by using the standard method with some modifications (Epa, 1989). Cultures were carried out in test tubes. Each tube was inoculated with a volume of *M. aeruginosa* in the exponential growth phase to make an initial density of 0.25×10^6 cells mL^{-1}. *M. aeruginosa* cultures were added to the test tubes at different concentrations (0 [control], 50, 100, and 200 µg/mL of the rich polyphenol extract). The cultures were incubated at 28°C, and illuminated in a 12h/12h light-dark cycle with fluorescent tubes (2000 lx m^{-2} s^{-1}). After initiation of the experiment, the cell density of each culture was measured (3, 4, 5, 6, and 7 days) using a Malassez hemocytometer. The inhibition efficiency was then calculated using the formula:

$$(IE)(\%) = [(N_o - N)/N_o] \times 100$$

where N and N_o (cells mL^{-1}) are the cell densities in the treatment and control cultures, respectively.

Statistical Analysis

Data were presented as means ± standard deviation. The statistical significance was confirmed by analysis of variance (ANOVA), followed by Tukey's test for pairwise comparison of means. A *P*-value of <0.05 was considered significant.

Results and Discussion

Phytochemical constituents

The phytochemical tests showed the presence of various secondary compounds in this species such as phenolics, flavonoids, anthraquinones, glycosides, and saponins (**Figure 2**). These results are similar to the previous study on the phytochemistry of *L. octovalvis* by Aung & Chaw (2019). The rich phenolic extract from the species was chosen to test against *M. aeruginosa* growth since phenolics are the largest group of phytochemicals and are responsible for controlling algal blooms.

Extraction of polyphenols

The effect of solvent type

The effect of solvent type (water-diluted ethanol, acetone, methanol, and water) on the total polyphenol content was evaluated with a solvent/material ratio of 20 mL g^{-1}, a water bath temperature of 30°C, and an extraction time of about 20min. The total phenol contents of the *L. octovalvis* extracts using different solvents are presented in **Table 1**.

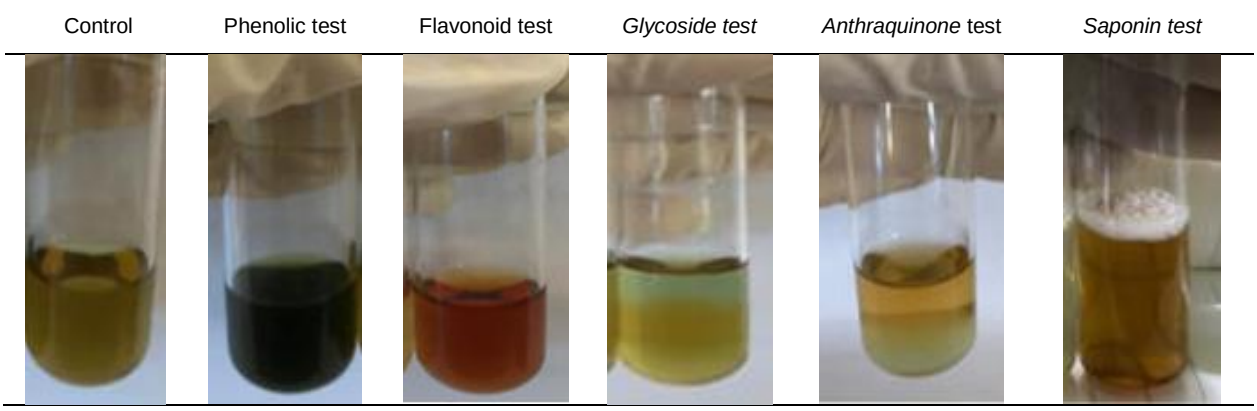

Figure 2. Results of the phytochemicals tests on the *L. octovalvis* extract

Table 1. Effect of solvent type on the TP content

Solvent	TP (mgGAE g^{-1})
Aqueous acetone (70%, v/v)	$146.26^{a} \pm 4.77$
Aqueous methanol (70%, v/v)	$120.78^{b} \pm 3.27$
Aqueous ethanol (70%, v/v)	$99.43^{c} \pm 1.78$
Water	$46.34^{d} \pm 0.55$

Note: Values in a column with different superscripts are significantly different (P <0.05)

The results revealed that acetone, methanol, and ethanol were better solvents for phenolic extraction compared to water only, with the high TP values of 146.26 ± 4.77, 120.78 ± 3.27, and 99.43 ± 1.78mg GAE g^{-1}, respectively. Polar solvents such as methanol, ethanol, acetone, and water were commonly used to extract polyphenols from plant samples (Yakob *et al.*, 2012; Oreopoulou *et al*., 2019; Trang *et al*., 2022) and *L. octovalvis (*Yakob *et al.*, 2012) in previous studies. Because the extraction process using acetone water obtained the highest polyphenol value, this solvent was determined to be best suitable for extracting polyphenol compounds from *L. octovalvis.*

Effect of the concentration of acetone

The data in **Table 2** show the yields of total polyphenols obtained from *L. octovalvis* using the acetone-water mixture and anhydrous acetone. *In our observations, t*he increase in acetone from 30% to 70% (v/v) resulted in a considerable increase in the TP value. However, the phenolic total value decreased significantly when the acetone concentration was increased to 100%. A similar pattern of TP values was reported by Nayak *et al.* (2015) when they performed extractions from *Citrus sinensis* peels using acetone-water as the extraction solvent. Based on these results, the solvent system of acetone and water (70:30, v/v) was found to be the best solvent to extract polyphenol compounds from *L. octovalvis* and was chosen for further study.

Effect of the solvent-to-material ratio

We investigated how the solvent-to-material ratio (15, 20, 30, and 40 mL g^{-1}) affected the extraction of polyphenols using an acetone concentration of 70% (v/v) and an extraction time of 20min at 30°C. The results presented in **Table 3** indicate that the TP increased from

Table 2. Effect of concentrations of acetone on the TP content

Concentration (%, v/v)	TP (mg GAE g^{-1})
30	$60.41^{d} \pm 1.90$
50	$84.53^{b} \pm 4.75$
70	$154.53^{a} \pm 4.60$
100	$71.05^{c} \pm 2.21$

Note: Values in a column with different superscripts are significantly different (P <0.05)

Table 3. Effect of material/solvent ratios on the TP content

Solvent-to-material ratio (mL g^{-1})	TP (mg GAE g^{-1})
15	$89.42^{b} \pm 0.90$
20	$151.84^{a} \pm 6.68$
30	$153.52^{a} \pm 3.97$
40	$160.32^{a} \pm 5.02$

Note: Values in a column with different superscripts are significantly different (P <0.05).

89.42 ± 0.90 to 151.84 ± 6.68 mg GAE g^{-1} when the solvent-to-solid ratio was increased from 15 to 20 mL g^{-1}. However, the results of the one-way analysis of variance showed that the material/solvent ratios were not significantly different among the ratios studied (P <0.05) when the solvent-material ratio increased from 20 to 40 mL g^{-1} in the tests. The solubility of the materials in the solid layer improves as the solvent content rises. In the extraction of polyphenols, this effect has already been investigated in previous studies (Yang *et al.*, 2009; Nayak *et al.*, 2015). By using a higher ratio of solvents, we could get more and more other constituents in plants, so unexpected substances would prevent the dissolution of polyphenols. As such, we chose a ratio of 20 mL/g in a continuous experiment.

Effects of the *L. octovalvis* extract on *M. aeruginosa* growth

The inhibitory effects (IE) of different concentrations of the extracted solution on the growth of *M. aeruginosa* are shown in **Figure 3a**. *In the control tube, the cell density increased from 0.25* x 10^6 cells mL^{-1} to 4.26 ± 0.15 x 10^6 cells mL^{-1} after 7 days of testing. All tested concentrations of the extract had a positive inhibitory effect on *M. aeruginosa* growth. As shown in **Figure 3a**, there was not a significant difference in the cell densities between the two tested concentrations of 50 and 100 mg/mL after 3 days of testing (P <0.05). However, differences in algicidal activity were observed over the next days of the experiment. Cell density decreased markedly after 5 days of testing at the concentrations of 100 and 200 mg mL^{-1}. The density of cells on day 6 of the 100 and 200 concentrations decreased before a slight increase on day 7, possibly because the number of previously dead algal cells was no longer recorded when measured, so only the number of live cells was measured. The IE values of the *L. octovavis* extracts are presented in **Figure 3b**. The inhibition efficiency after after 7 days increased from 40.71 to 81.56%, for the concentrations ranging from 50 to 200 µg mL^{-1}. The increase of IE at high concentrations can be explained by the presence of algicidal compounds in the extract.

Plant extracts containing polyphenol-rich compounds can behave like potential algicides. The effective concentrations of extracts have varied in previous reports. For example, rice straw extract showed potent algicidal activity at low concentrations ranging from 0.1 to 10 µg mL^{-1} (Park *et al.*, 2006). *Eupatorium fortune* extracts affected *M. aeruginosa* at high concentrations from 200-500 µg mL^{-1} after 10 days of treatment, with IE values ranging from 49.0% and 95.5% (Nga *et al.*, 2017). Ethyl acetate extracts and methanol extract of Sugi bark contained high levels of flavanols, and showed IE values of over 60% at concentrations of 5 mg mL^{-1} after 8 days (Suzuki *et al.*, 2018). Both ethanolic and methanolic extracts of *Bidens pilosa*, in contrast to the other plant extracts, exhibited high inhibitory effects on *M. aeruginosa* growth at a concentration of 500 mg L^{-1} (Van Nguyen *et al*., 2019).

Conclusions

L. octovalvis is rich in polyphenols with a total polyphenol content value of 149.22 ± 0.96 mg GAE/g. The inhibitory efficiency of *L. octovalvis* extract on *M. aeruginosa* growth increased from 40.71 to 81.56% after 7 days of testing when the concentration of the extract varied from 50-200 µg mL^{-1}.

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References

Aung L. W. & Chaw D. K. E. (2019). Study on Morphology, Anatomy, Preliminary Phytochemical Test, Nutritional Values and Antimicrobial Activities of leaves of *Ludwigia octovalvis* (Jacq.) Raven. Dagon University Commemoration of 25th Anniversary Silver Jubilee Research Journal. 9(2): 321-327.

Baky M. H., Elgindi M. R., Shawky E. M. & Ibrahim H. A. (2022). Phytochemical investigation of Ludwigia adscendens subsp. diffusa aerial parts in context of its biological activity. BMC Chemistry. 16(1): 112.

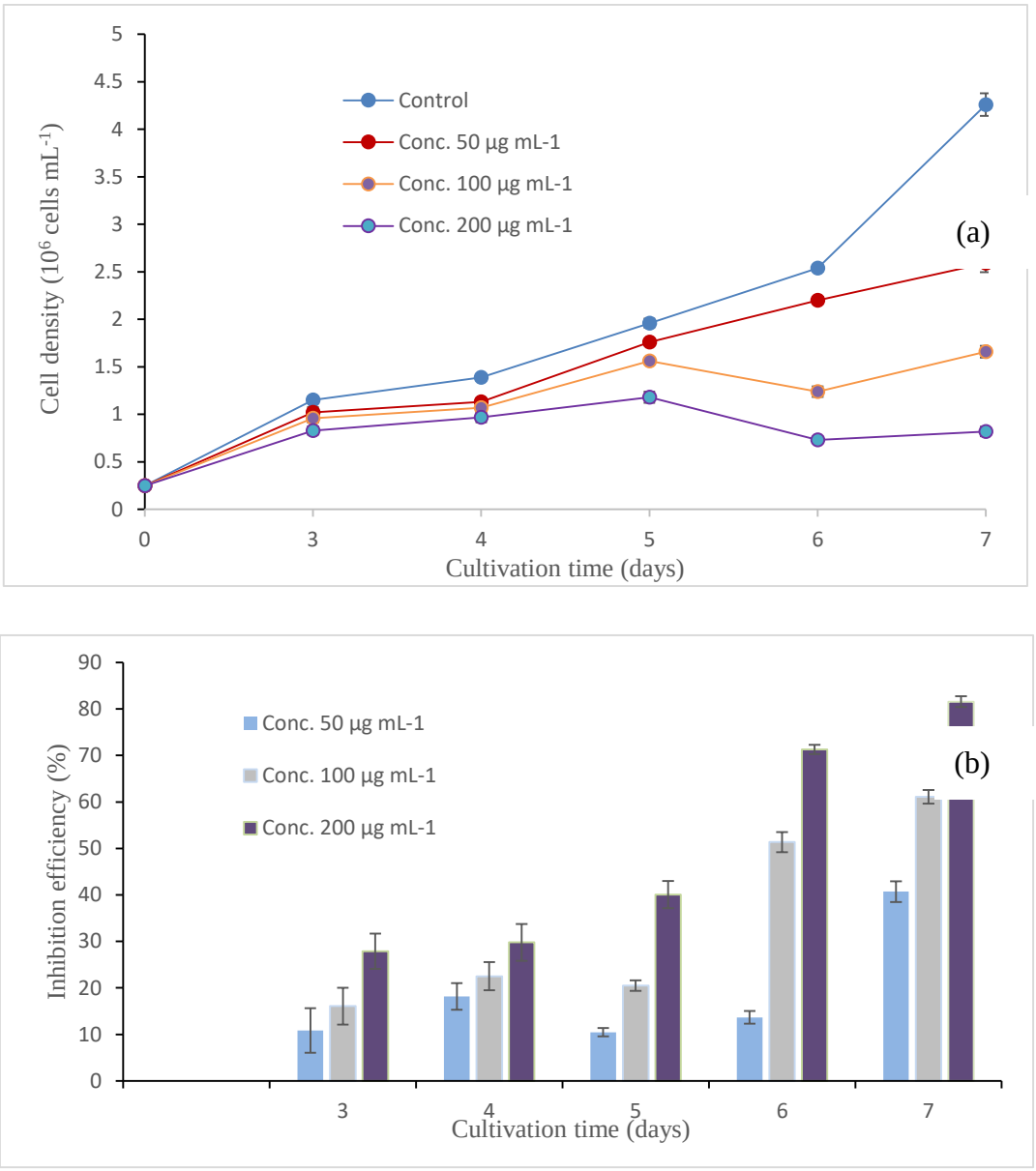

Figure 3. Effects of the *L. octovalvis* extract on *M. aeruginosa* growth in terms of (a) cell density and (b) inhibition efficiency

Barrett P., Littlejohn J. & Curnow J. (1999). Long-term algal control in a reservoir using barley straw. In: Biology, Ecology and Management of Aquatic Plants. Springer: 309-313.

Chen J., Hoch P. C., Raven P. H., Boufford D. A. & Wagner W. L. (2007). Section 29 Onagraceae. In: Wu Z. Y., Raven P. H. & Hong D. Y. (Eds). Flora of China vol. 13. Science Press, Beijing and Missouri Botanical Garden Press, St. Louis: 400-404.

Ding W.-X., Shen H.-M., Shen Y., Zhu H.-G. & Ong C.-N. (1998). Microcystic cyanobacteria causes mitochondrial membrane potential alteration and reactive oxygen species formation in primary cultured rat hepatocytes. Environmental Health Perspectives. 106(7): 409-413.

Fan J., Hobson P., Ho L., Daly R. & Brookes J. (2014). The effects of various control and water treatment processes on the membrane integrity and toxin fate of cyanobacteria. Journal of Hazardous Materials. 264: 313-322.

Gross E. M., Meyer H. & Schilling G. (1996). Release and ecological impact of algicidal hydrolysable polyphenols in Myriophyllum spicatum. Phytochemistry. 41(1): 133-138.

Harborne A. (1998). Phytochemical methods a guide to modern techniques of plant analysis. Springer Science and Business Media.

Huang H., Xiao X., Ghadouani A., Wu J., Nie Z., Peng C., Xu X. & Shi J. (2015). Effects of natural flavonoids on

photosynthetic activity and cell integrity in Microcystis aeruginosa. Toxins. 7(1): 66-80.

Jančula D. & Maršálek B. (2011). Critical review of actually available chemical compounds for prevention and management of cyanobacterial blooms. Chemosphere. 85(9): 1415-1422.

Karan V., Vitorović S., Tutundžić V. & Poleksić V. (1998). Functional enzymes activity and gill histology of carp after copper sulfate exposure and recovery. Ecotoxicology and Environmental Safety. 40(1-2): 49-55.

Meng P., Pei H., Hu W., Liu Z., Li X. & Xu H. (2015). Allelopathic effects of *Ailanthus altissima* extracts on *Microcystis aeruginosa* growth, physiological changes and microcystins release. Chemosphere. 141: 219-226.

Moghaddam Z. (2012). Effects of solvent type on phenolics and flavonoids content and antioxidant activities in Onosma dichroanthum Boiss. Journal of Medicinal Plants Research. 6(28): 4481-4448.

Mohammedi Z. & Atik F. (2011). Impact of solvent extraction type on total polyphenols content and biological activity from Tamarix aphylla (L.) Karst. International Journal of Pharma and Bio Sciences. 2(1): 609-615.

Nakagawa M., Takamura Y. & Yagi O. (1987). Isolation and characterization of the slime from a cyanobacterium, *Microcystis aeruginosa* K-3A. Agricultural and Biological Chemistry. 51(2): 329-337.

Nakai S., Inoue Y. & Hosomi M. (2001). Algal growth inhibition effects and inducement modes by plant-producing phenols. Water Research. 35(7): 1855-1859.

Nayak B., Dahmoune F., Moussi K., Remini H., Dairi S., Aoun O. & Khodir M. (2015). Comparison of microwave, ultrasound and accelerated-assisted solvent extraction for recovery of polyphenols from Citrus sinensis peels. Food Chemistry. 187: 507-516.

Nga P. T., Dien P. H., Quyen N. V., Thuong T. H., Quynh L. T. P., Dat N. T., Thuy D. T. & Kim D. D. (2017). Inhibitory effect of different *Eupatorium fortunei* Turcz extracts on the growth of *Microcystis aeruginosa*. Vietnam Journal of Science and Technology. 55(4C): 103.

Nishiwaki-Matsushima R., Ohta T., Nishiwaki S., Suganuma M., Kohyama K., Ishikawa T., Carmichael W. W. & Fujiki H. (1992). Liver tumor promotion by the cyanobacterial cyclic peptide toxin microcystin-LR. Journal of Cancer Research and Clinical Oncology. 118(6): 420-424.

Oh H.-M., Lee S. J., Jang M.-H. & Yoon B.-D. (2000). Microcystin production by *Microcystis aeruginosa* in a phosphorus-limited chemostat. Applied and environmental Microbiology. 66(1): 176-179.

Oreopoulou A., Tsimogiannis D. & Oreopoulou V. (2019). Extraction of polyphenols from aromatic and medicinal plants: an overview of the methods and the effect of extraction parameters. In: Watson R. R. (2019). Polyphenols in Plants, Isolation, Purification and Extract Preparation (2nd ed.). Academic Press: 243-260.

Park M. H., Han M. S., Ahn C. Y., Kim H. S., Yoon B. D. & Oh H. M. (2006). Growth inhibition of bloom-forming cyanobacterium Microcystis aeruginosa by rice straw extract. Letters in Applied Microbiology. 43(3): 307-312.

Pillinger J., Cooper J. & Ridge I. (1994). Role of phenolic compounds in the antialgal activity of barley straw. Journal of Chemical Ecology. 20(7): 1557-1569.

Rao P., Bhattacharya R., Pant S. & Bhaskar A. (1995). Toxicity evaluation of in vitro cultures of freshwater cyanobacterium Microcystis aeruginosa: I. Hepatotoxic and histopathological effects in rats. Biomedical and Environmental Sciences: BES. 8(3): 254-264.

Sankar R., Prasath B. B., Nandakumar R., Santhanam P., Shivashangari K. S. & Ravikumar V. (2014). Growth inhibition of bloom forming cyanobacterium *Microcystis aeruginosa* by green route fabricated copper oxide nanoparticles. Environmental Science and Pollution Research. 21(24): 14232-14240.

Shao J., Li R., Lepo J. E. & Gu J.-D. (2013). Potential for control of harmful cyanobacterial blooms using biologically derived substances: problems and prospects. Journal of Environmental Management. 125: 149-155.

Shawky E. M., Elgindi M. & Hassan M. M. (2023). Phytochemical and biological diversity of genus Ludwigia: A comprehensive review. Egyptian Russian University Research Journal. 2(3): 447-474.

Singleton V. L. & Rossi J. A. (1965). Colorimetry of Total Phenolics with Phosphomolybdic-Phosphotungstic Acid Reagents. Am J Enol Vitic. 16(3): 144-158.

Smida I., Sweidan A., Souissi Y., Rouaud I., Sauvager A., Torre F., Calvert V., Le Petit J. & Tomasi S. (2018). Anti-acne, antioxidant and cytotoxic properties of Ludwigia peploides leaf extract. International Journal of Pharmacognosy and Phytochemical Research. 10(7): 271-278.

Sofowora A. (1996). Medicinal plants and traditional medicine in Africa. Karthala.

Suzuki Y., Saijo H., Takahashi K., Kofujita H. & Ashitani T. (2018). Growth-inhibitory components in Sugi (*Cryptomeria japonica*) extracts active against Microcystis aeruginosa. Cogent Environmental Science. 4(1): 1466401.

Tebaa L., Douma M., Tazart Z., Manaut N., Mouhri K. & Loudiki M. (2017). Algicidal effects of Achillea ageratum L. and Origanum compactum Benth. plant extracts on growth of Microcystis aeruginosa. Applied Ecology and Environmental Research. 15(4): 719-728.

Trang T. T. T., Ha L. T. N., Ai D. T. T., Hien N. T., Tram N. T. T. & Huyen V. T. (2022). Phytochemical Analysis and Antioxidant and Alpha-glucosidase Inhibitory Activities of the Stem Bark of Dialium cochinchinense Pierre. Vietnam Journal of Agricultural Sciences. 5(1): 1375-1388.

US. EPA (1989). Green Algal, Selenastrum capricornutum, growth test method 1003.0. In: Weber C. I., Peltier W. H., Norberg-King T. J., Horning W. B., Kessler F. A., Menkedick J. R., Neiheisel T. W., Lewis P. A., Klemm D. J., Pickering Q. H., Robinson E. L., Lazorchak J. M., Wymer L. J. & Freyberg R. W. (Eds.) (1989). Short-term methods for estimating the chronic toxicity of effluents and receiving waters to freshwater organisms (2nd ed.). U.S. Environmental Protection Agency, Cincinnati, Ohio.

Van Nguyen Q., Tran T. H., Pham T. N., Van Thuoc D., Cao V. D. & Boo K. H. (2019). Inhibitory effects of Bidens pilosa plant extracts on the growth of the bloom-forming alga Microcystis aeruginosa. Water, Air and Soil Pollution. 230: 1-16.

Wu S.-J., Ng L.-T., Wang G.-H., Huang Y.-J., Chen J.-L. & Sun F.-M. (2010). Chlorophyll a, an active anti-proliferative compound of *Ludwigia octovalvis*, activates the CD95 (APO-1/CD95) system and AMPK pathway in 3T3-L1 cells. Food and Chemical Toxicology. 48(2): 716-721.

Yakob H. K., Sulaiman S. F. & Uyub A. M. (2012). Antioxidant and antibacterial activity of Ludwigia octovalvis on Escherichia coli O157: H7 and some pathogenic bacteria. World Applied Sciences Journal. 16: 22-29.

Yakob H. K., Uyub A. M. & Sulaiman S. F. (2015). Immune-stimulating properties of 80% methanolic extract of *Ludwigia octovalvis* against Shiga toxin-producing E. coli O157: H7 in Balb/c mice following experimental infection. Journal of Ethnopharmacology. 172: 30-37.

Yang L., Jiang J. G., Li W. F., Chen J., Wang D. Y. & Zhu L. (2009). Optimum extraction process of polyphenols from the bark of *Phyllanthus emblica* L. based on the response surface methodology. Journal of Separation Science. 32(9): 1437-1444.

Zhang T.-T., Zheng C.-Y., Hu W., Xu W.-W. & Wang H.-F. (2010). The allelopathy and allelopathic mechanism of phenolic acids on toxic Microcystis aeruginosa. Journal of Applied Phycology. 22(1): 71-77.