Assessment of the Effects of Four Plant Species Used in Mexican Traditional Medicine Against *Microcystis aeruginosa*

René Solano Jiménez (✉ ibq.rene.solano@hotmail.com)

Instituto Mexicano de Tecnología del Agua: Instituto Mexicano de Tecnología del Agua
<https://orcid.org/0000-0002-2305-0815>

Sofía Esperanza Garrido Hoyos

Mexican Institute of Water Technology: Instituto Mexicano de Tecnología del Agua

Ana María Sandoval Villasana

Mexican Institute of Water Technology: Instituto Mexicano de Tecnología del Agua

Maria Luisa Villareal

Universidad Autónoma del Estado de Morelos Centro de Investigación en Biotecnología: Universidad Autónoma del Estado de Morelos Centro de Investigación en Biotecnología

José Roberto Villagómez Ibarra

Universidad Autónoma del Estado de Hidalgo - Instituto de Ciencias Básicas e Ingeniería

Research Article

Keywords: Anti-algal activity, algaecide, algistatic effect, crude extracts, cyanobacterial blooms, herbal medicine plants, inhibition mechanisms, *Microcystis aeruginosa*

Posted Date: July 23rd, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-628419/v1>

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Abstract

Microcystis aeruginosa is one of the main harmful bloom-forming cyanobacteria species worldwide. Plant extracts are considered an appropriate practice to obtain effective, economical and sustainable algaecides. For the first time, the effects of *Argemone mexicana*, *Bougainvillea spectabilis*, *Larrea tridentata* and *Tradescantia zebrina* on the growth of *M. aeruginosa* in solid and liquid media were investigated by measuring cell density, Chl-a concentration, malondialdehyde (MDA), proteins and nucleotides. Total polyphenol content was quantified to reveal the nature of inhibition. The results in solid medium showed that the methanolic extract of *L. tridentata* had the greatest effect, showing an inhibition zone of 28.67 ± 0.58 mm. In liquid medium, the methanolic extract of *L. tridentata* again showed the greatest effects on the cell density of *M. aeruginosa* (93.80%, $p < 0.0001$), followed by *B. spectabilis* (87.66%, $p < 0.0001$) and *A. mexicana* with (87.11%, $p < 0.001$), with $EC_{50} = 13.63, 20.27$ and 24.47 mg/L, respectively. *L. tridentata* also showed a decrease of Chl-a of 91.51% ($P < 0.0001$), MDA increased 6.42 times, and proteins and nucleotides 2.09 and 2.12 times in relation to the control, respectively. *L. tridentata* showed the highest total polyphenol content, 363.51 mgGAEq/gDE. These results indicate that the decrease in photosynthetic capacity and lipid peroxidation are the most probable mechanisms of action of this species against *M. aeruginosa*.

Introduction

Cyanobacterial blooms in eutrophic lakes are a global problem that has been intensifying since the last few decades (Huisman et al., 2018). Recent studies on cyanobacterial abundance and hydroclimatic changes over the past millennium show that cyanobacterial abundance has been significantly higher in warmer periods, including the current warm period (1900-present), and may continue to increase in coming years in subtropical water bodies as a consequence of global warming (Yan et al., 2020).

Recent research has focused on the development of plant-derived algaecides for the control of cyanobacterial blooms. There is evidence that extracts from a wide range of aquatic macrophyte species possess inhibitory effects on cyanobacteria, such as extracts from *Ceratophyllum demersum* and *Potamogeton pectinatus* (Ghobrial et al., 2015; Wang et al., 2015), *Potamogeton cristatus*, *Potamogeton maackianus*, *Potamogeton lucens*, *Vallisneria spirulosa* and *Hydrilla verticillata* (Wang et al., 2015), which are highly harmful to *M. aeruginosa* and *Oscillatoria tenuis*. Even some species such as *Lemna trisulca* besides inhibiting the growth of *Anabaena flos-aquae*, also degrades its neurotoxin, anatoxin-a (Kaminski et al., 2014; Kaminski et al., 2015).

Medicinal plants have long been used in Mexican traditional medicine, and there is increasing research on the use of plants to control algal growth, but the available information on the use of Mexican medicinal plants for the control of *M. aeruginosa* is practically nil. However, it has been observed that species such as *Achillea ageratum* L. and *Origanum compactum* (Tebaa et al., 2017), *Dracontomelon duperreanum* (Wang et al., 2016) and some compounds such as the terpenoid artemisinin from *Artemisia annua* (Ni et al., 2012); ginkgolic acids present in *Ginkgo biloba* (Zhang et al., 2013a), neo-przewaquinone

A isolated from *Salvia miltiorrhiza* (Zhang et al., 2013b), have shown potent effect on *M. aeruginosa*. Currently, allelochemicals with anti-cyanobacterial activity identified include, phenolic acids, fatty acids, tannic acid, lactones, terpenoids, alkaloids, flavonoids and sulfides (Tan et al., 2019).

The objective of this study was to evaluate the algicidal effects of four species used in mexican traditional medicine, *A. mexicana*, *B. spectabilis*, *L. tridentata* and *T. zebrina*, on the toxic bloom-forming cyanobacterium *M. aeruginosa* and to identify possible mechanisms of action, focusing the experiments on (i) growth inhibition on solid agar and (ii) growth inhibition in liquid medium, effects on Chl-a, MDA, protein and nucleotide content.

Materials And Methods

Plant material

In our study, 4 plant species used in mexican traditional medicine were selected for their availability in accessible areas and for their antibacterial activities. The species were collected between December 2018 and March 2019, in different localities of the Mexican Republic and identified in the HUMO herbarium of the Biodiversity and Conservation Research Center (CIByC) of the Autonomous University of the State of Morelos (UAEM), generating a folio number for each species (Table 1). All species were washed with tap water and then rinsed with distilled water to remove debris and dried at room temperature. Subsequently, the plants were pulverized separately to use their powder in the extraction process.

Table 1 Geographic location of plant species collected in Mexico

Species	Location	Geographical location	HUMO Foil
<i>A. mexicana</i> L.	Cuauchichinola, Morelos	18.893975, -99.168232	38871
<i>B. spectabilis</i> Willd.	Jiutepec, Morelos	22.153655, -100.886267	38872
<i>L. tridentata</i> (DC.) Coville.	Los Gómez, San Luis Potosí	18.670007, -99.375895	38873
<i>T. zebrina</i> Bosse.	Jiutepec, Morelos	18.884940, -99.160087	38874

Preparation of extracts

Half of each powdered plant was mixed with 6 L of absolute ethyl alcohol (99.5%, Sigma-Aldrich) and absolute methyl alcohol (99.8%, Sigma-Aldrich), respectively, and left to macerate for 7 days at room temperature (25-29°C). The supernatants were filtered under reduced pressure to remove plant material through Whatman No. 1 paper (pore size 11 µm) and concentrated on a rotary evaporator at 39-40°C. Concentrated liquid extracts were transferred to glass vials and frozen at -80 °C for freeze-drying. All extracts were stored refrigerated (4 °C±0.1) until use. Table 2 shows the yields of the extracts of the plant species used in this study.

Table 2 Yields of ethanolic and methanolic extracts of different plant species

Species	Disolvent	Plant quantity (g)	Moist extract (mL)	Dried extract (g)	Yield (%)
<i>A. mexicana</i>	Ethanol	640	70	37.93	5.92
	Methanol	640	140	80.56	12.58
<i>B. spectabilis</i>	Ethanol	320	280	20.76	6.48
	Methanol	320	280	50.28	15.71
<i>L. tridentata</i>	Ethanol	1,470	385	166.02	11.29
	Methanol	1,470	420	241.21	16.40
<i>T. zebrina</i>	Ethanol	150	105	5.11	3.40
	Methanol	150	140	17.58	11.72

Determination of total polyphenols

The concentration of total polyphenolic compounds in the extracts was determined using the Folin-Ciocalteu method. A standard curve of gallic acid (with concentrations of 0, 1, 2.5, 5, 7.5, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 and 20 mg/L) was prepared and the equation $y=0.1186x+0.0077$ with a coefficient of determination ($R^2=0.9961$) was obtained. Subsequently, 2 mg of each extract was suspended in 3 mL of methanol and brought to 10 mL with distilled water. Next, 500 μ L of each of these solutions were taken and mixed with 250 μ L of F-C 1N reagent (Sigma-Aldrich), shaken for 5 min and 1,250 μ L of 20% Na_2CO_3 (J. T. Baker) was added. The samples were allowed to stand for 2 h and absorbances were measured at 760 nm using the UV-Vis 1800 spectrophotometer (Shimadzu). The values of x in the above equation were substituted for the values of the absorbances obtained and the results were recorded as mg gallic acid equivalent/g dry extract (mgGAEq/gDE).

Cyanobacteria cultivation

The cyanobacterial species *M. aeruginosa* was provided by the Bacteriology Laboratory of the FES-I (Facultad de Estudios Superiores Iztacala) of UNAM (Universidad Nacional Aut3noma de M3xico). *M. aeruginosa* was grown in BG-11 medium, with a light intensity of 57 $\mu\text{mol photons m}^{-2}\text{s}^{-1}$, at $28\pm1^\circ\text{C}$ and with a 12:12 hour light:dark photoperiod. The culture was shaken three times daily for 2 weeks and was used when *M. aeruginosa* reached the exponential growth phase, which was determined by measuring cell density every day for 18 days.

Evaluation of algaecidal effects in solid medium

To evaluate the inhibitory effects of ethanolic and methanolic extracts of the plant species, the procedure established by Zerrifi et al., 2019, with some modifications, was followed. The algaecidal activity of the extracts was evaluated using the agar diffusion technique in Petri dishes. 50 mg of each extract was resuspended in 1 mL of methanol. Filter paper discs (Whatman No. 1) of 9 mm diameter were

impregnated with 20, 60 and 100 µL (1, 3 and 5 mg) of the different extracts, copper sulfate (J. T. Baker) and methanol impregnated discs were also used as positive and negative controls and air dried. The discs were placed on BG11 medium with 3% bacteriological agar and 500 µL of previously inoculated *M. aeruginosa*. All plates were sealed with parafilm and exposed to the same *M. aeruginosa* culture conditions as above. Inhibitory effects were measured by the diameter value of the inhibition zone after 9 days of *M. aeruginosa* growth.

Measurements of cell density

To analyze the concentration-dependent effects of the methanolic plant extracts on the growth of *M. aeruginosa*, the different initial concentrations of extracts were used. 3 mL of the exponentially growing *M. aeruginosa* culture was inoculated into 250 mL flasks and 100 mL of BG-11 and allowed to acclimate for 7 days. Various doses of solution of each extract (previously resuspended in 0.2% dimethyl sulfoxide) were added to the cultures to obtain final concentrations of 10, 25, 50, 50 and 75 mg/L. The initial density of *M. aeruginosa* was approximately 1×10^6 cells/mL. Cultures were allowed to grow for 12 days under the same conditions as described above. No extract was added to the control groups and each experiment included treatments in triplicate. At two-day intervals, *M. aeruginosa* growth was monitored by counting cells with an optical microscope and a hemocytometer. The inhibition ratio (IR) was used for expressing the effects of the extracts, which was defined as follows:

$$IR(\%) = \left(1 - \frac{T}{C}\right) \times 100$$

Where T and C refer to cell density of treatment and control, respectively.

Effective concentration 50 (CE50)

The 50% effective concentration (EC₅₀) defined as the concentration at which the IR is 50% on the inhibition curve, was calculated at 12-days and based on the cell density using linear interpolation analysis.

Quantification of Chl-a

Based on the results obtained in the previous sections (see Results), the *L. tridentata* extract was selected for further analysis of its mechanisms of action on *M. aeruginosa*. Chlorophyll-a concentration [Chl-a] was measured using UV-Vis 1800 spectrophotometer (Shimadzu) and calculated with reference to the standard method described by Hein (2017). An appropriate volume of each culture was passed through a glass fiber filter under reduced pressure, the filters were recovered and 10 mL of 90% acetone was added. Samples were triturated with a tissue homogenizer and stored in the dark for 24 h at 4 °C. The samples were then centrifuged for 20 min at 3000 rpm, the supernatants were measured at 664 and 750 nm, acidified with 0.1 mL of 0.1 N HCl (36.6-38%, J. T. Baker), allowed to react for 90 s and measured again at

absorbances 665 and 750 nm. The Chl-a concentration (mg/m³) was calculated according to the following equation:

$$[\text{Chl a}] = \frac{26.7(664_b - 665_a) \times V_1}{V_2 \times L}$$

where 664_b and 665_a: values of the absorbances before and after acidification, V₁: volume of the extract in liters, V₂: volume of the sample in m³ and L: light path in the cuvette in cm. The results were transformed to mg/L.

Lipid peroxidation

Lipid peroxidation was reflected in terms of malondialdehyde MDA concentration [MDA] in cells and was determined according to the work of Li et al (2016), with slight modifications. Appropriate volumes of each sample were collected and centrifuged at 4,000 rpm for 10 min. The supernatant was carefully removed, the cell pellets were resuspended with 5 mL of 10% (w/v) trichloroacetic acid (≥99%, Sigma Aldrich) and centrifuged at 12,000 rpm for 10 min. Then, 2 mL of the supernatant was mixed with 2 mL of 0.6% thiobarbituric acid (≥98%, Sigma Aldrich) (in 10% trichloroacetic acid solution) and incubated in water at 95 °C for 30 min. Samples were cooled in water at room temperature and centrifuged again at 12,000 rpm for 10 min. The absorbances of the supernatants were measured at 532, 600 and 450 nm, taking as blank a mixture of 2 mL ultrapure water and 2 mL 0.6% thiobarbituric acid. The MDA level (μmol/L) was calculated according to eq:

$$[\text{MDA}] = 6.45 \times (\text{OD}_{532} - \text{OD}_{600}) - 0.56 - \text{OD}_{450}$$

Quantification of proteins and nucleotides

Proteins and nucleotides have been documented to have strong UV absorption at 280 and 260 nm, respectively, and can be easily detected using a UV-Vis spectrophotometer. This method is widely used to determine membrane integrity. Five mL of *M. aeruginosa* culture treated with different concentrations of *L. tridentata* were taken and filtered with 0.22 μm Millipore filter to remove cell biomass. The supernatants were measured at 260 and 280 nm using the UV-Vis 1800 spectrophotometer (Shimadzu). The OD ratio between the experimental group and the control group was used to evaluate the level of protein and nucleotide release (Zhang et al., 2012).

Statistical analysis

Analyses of the data were performed using GraphPad Prism 8.0.1 software. All experiments were performed in three replicates. Means and standard deviation (SD) were calculated and presented. Data from different treatments were compared to the control, to determine statistical significance using the

ANOVA test and Tukey test. Differences were considered to be significant at $p<0.05(*)$, $p<0.01(**)$, $p<0.001(***)$ and $p<0.0001(****)$.

Results

Concentration of total polyphenols in the extracts

The results of total polyphenol quantification of ethanolic and methanolic plant extracts expressed in mg gallic acid equivalents/g dry extract (mgGAEq/gDE) are presented in Fig. 1. *L. tridentata* presented the highest concentrations (266.49 ± 12.29 , 363.51 ± 13.63 , respectively). In *A. mexicana* the concentrations were 112.97 ± 15.41 and 150.60 ± 24.21 . *B. spectabilis* showed slightly lower concentrations, 99.26 ± 4.17 and 100.67 ± 7.72 . The ethanolic and methanolic extracts of *T. zebrina* showed the lowest concentrations, 33.18 ± 0.72 and 52.48 ± 0.57 , respectively.

Algaecide tests on solid agar

After 9 days of growth, inhibition halos were measured and the results obtained are presented in Fig. 2a and b. All methanolic extracts were slightly more effective than ethanolic extracts. The most important effects were observed with the methanolic extract of *L. tridentata*, with 5 mg presented an inhibition zone of 28.67 ± 0.58 mm ($p<0.0001$). The methanolic extracts of *A. mexicana* and *B. spectabilis* exhibited similar results in the three concentrations analyzed, with 5 mg, showed inhibition halos of 20.33 ± 1.53 and 21.00 ± 1.00 mm ($p<0.0001$), respectively. The smallest effects were observed with *T. zebrina*, with an average inhibition of 12.00 ± 1.00 mm ($p<0.001$) with 5 mg. Methanol used as a negative control showed no effect, while CuSO_4 presented the greatest algaecidal effect, 42.00 ± 1.00 mm ($p<0.0001$).

Effects of methanolic extracts on the growth of *M. aeruginosa*

The results of growth inhibition of *M. aeruginosa* in liquid medium are presented in Fig. 3 and 4. *L. tridentata* recorded the highest inhibitory effects: from day 8, a significant decrease in the cell density of *M. aeruginosa* was observed in the four treatments (10, 25, 50 and 75 mg/L), reaching inhibitions of 44.96% ($p<0.01$), 55.83% ($p<0.001$), 68.66% ($p<0.01$) and 81.10% ($p<0.001$). By day 10, the inhibitions recorded were 29.69% ($p<0.05$), 62.19%, 76.48 and 87.52% ($p<0.001$). However, the greatest effects were obtained on day 12, where cell densities were 5.411, 2.656, 1.509 and 0.563×10^6 cells/mL, with inhibitions of 40.45% ($p<0.01$), 70.77%, 83.39 and 93.80% ($p<0.0001$), with respect to the control. In descending order, *B. spectabilis* and *A. mexicana* presented similar results. In the *B. spectabilis* group, on day 12 at 10, 25, 50 and 75 mg/L, cell densities were 5.367, 4.071, 1.854 and 1.097×10^6 cells/mL, with inhibitions of 39.63% ($p<0.01$), 54.20% ($p<0.05$), 79.14 ($p<0.001$) and 87.66% ($p<0.0001$). As for the group studied with *A. mexicana*, the treatments with 50 and 75 mg/L, showed a clear decrease in cell density from day 6 and until the end of the experiment, where the cell densities were 1.214 and 1.066×10^6 cells/mL, with inhibitions of 85.57% and 87.33%, in relation to the control ($p<0.001$). The lowest effects were presented with *T. zebrina*, since no concentration exceeded 25% inhibition, only significant inhibition

was recorded at 50 mg/L on day 10 and at 75 mg/L on day 12, where cell densities were 5.536 and 6.783x10⁶ cells/mL, reaching inhibitions of 18.68 and 20.94% in relation to the control (p<0.05).

Effective concentration 50 (EC₅₀)

Based on the results of the growth inhibition tests of *M. aeruginosa* in liquid medium, the extracts of *A. mexicana*, *B. spectabilis* and *L. tridentata* were chosen to calculate their EC_{50, 12 d} and evaluate their anti-cyanobacterial efficacy. The lowest value was obtained with the extract of *L. tridentata* with a value of 13.63 mg/L, followed by the extract of *B. spectabilis* with 20.27 mg/L and *A. mexicana* with 24.47 mg/L.

Effects of *L. tridentata* on the content of Chl-a, MDA, nucleotides and proteins in *M. aeruginosa*

Based on the results of the EC_{50, 12 d}, the methanolic extract of *L. tridentata* was selected to study its effects on the photosynthetic activity of expressed as Chl-a concentration [Chl-a], lipid peroxidation [MDA] and on the leakage of intracellular material (nucleotides and proteins) in *M. aeruginosa*. The results obtained are shown in Fig. 5. In Fig. 5a it can be seen that [Chl-a] in *M. aeruginosa* was significantly affected with increasing concentration and with exposure time to *L. tridentata*. In the control group, [Chl-a] showed a significant increase with prolongation of time, reaching a [Chl-a] = 3.029 mg/L at day 12. Compared to the control, all treatment groups showed a significant decrease in [Chl-a] from day 8. Particularly, at concentrations of 50 and 75 mg/L, [Chl-a] decreased from day 2 to the end of the experiment, where [Chl-a] = 0.544 and 0.257 mg/L were quantified, which meant decreases of 82.04% and 91.51%, with respect to the control (p<0.0001).

As shown in Fig. 5b, [MDA] in *M. aeruginosa* visibly increased as concentration increased and exposure time extended. The control group showed no significant change in [MDA] throughout the experiment. After 2 days of exposure, only a significant increase in [MDA] was observed at 75 mg/L (p<0.05). From day 8, [MDA] increased significantly at all concentrations of *L. tridentata*, with the exception of day 10 at 10 mg/L. However, it was at day 12 where the greatest effects were observed, since at 25, 50 and 75 mg/L [MDA] = 4.42, 5.82 and 6.61 µmol/g protein were quantified, which translated into 4.3-, 5.65- and 6.42-fold increases compared to the control (p<0.001, p<0.001 and p<0.0001, respectively).

Figs. 5c and 5d show nucleotide and protein leakage as a probable consequence of lipid peroxidation of *M. aeruginosa* cells caused by prolonged exposure to *L. tridentata* extract. The control groups showed no significant change in OD₂₆₀ and OD₂₈₀ values throughout the experiment. From day 4, *L. tridentata* induced a gradual concentration-dependent increase in DO₂₆₀ and DO₂₈₀ values. By day 12, DO₂₆₀ values of 1.87 and 2.13, respectively, were quantified in the groups treated with 50 and 75 mg/L *L. tridentata*. For DO₂₈₀ under the same conditions, the values were 1.89 and 2.22, respectively. These values were significantly higher compared to their control groups (p<0.01). These results together with those of MDA, indicate that, without any doubt, the extract of *L. tridentata*, provoked a very severe lipid peroxidation in the cells of *M. aeruginosa*.

Discussion

In Mexico, medicinal plants have been used since pre-Hispanic times and it is estimated that, of the 30,000 recorded plant species, approximately 3,000 to 5,000 are of medicinal use and 75 species have shown antibacterial activity (Villaseñor et al., 2016; Palma et al., 2017). Phytochemical analysis in *A. mexicana* and *B. spectabilis* reveal that leaves, stems and flowers are rich in phenolic acids, tannins, flavonoids, terpenoids, alkaloids. They possess antibacterial activity against 9 Gram(+) and 9 Gram(-) and 8 Gram(+) and 13 Gram(-) bacteria, respectively (Brahmachari et al., 2013; Ghogar et al., 2016; Abarca and Petricevich, 2018). *L. tridentata* is widely distributed in the arid zones of northern Mexico and can survive under extreme conditions of drought and frost. In addition, mainly due to its extensive range of allelopathic substances and secondary metabolites, it is one of the dominant species in the desert microphytic scrub community, displacing other species typical of this ecosystem, hence its common name "gobernadora" (Mata et al., 2018). More than 20 flavonoid aglycones, phenolic lignans, alkaloids, alkaloids, terpenoids and fatty acids have been identified in the leaves and stems of *L. tridentata* (Arteaga et al., 2005). *T. zebrina* leaves exhibit a high content of phenolic compounds, tannins and flavonoids and possess activity against 6 Gram(+) and 2 Gram(-) bacteria (Tan et al., 2014). The presence of these compounds and reports of their antibacterial activity, make extracts of these species good candidates for algaecidal agents.

In solid medium tests, the methanolic extracts of the four species showed the highest inhibitory effects, despite the fact that, in the case of *T. zebrina*, the ethanolic extract presented a higher polyphenolic content. These results suggest that methanolic extracts may possess other families of algaecidal compounds in addition to polyphenols, which act synergistically in the inhibition process. The methanolic extracts of *A. mexicana*, *B. spectabilis* and *L. tridentata* showed the greatest effects, causing inhibition zones of 20.33, 21.00 and 28.67 mm. Our results are similar to those reported by Zerifi et al., 2019, when they observed that extracts of *Laurencia pinnatifida* and *Bornetia secundiflora* generated inhibition zones of 17.33 ± 0.33 and 27.33 ± 0.33 mm in *M. aeruginosa* culture after 7 days of growth.

In liquid medium, methanolic extracts of the four plant species possess statistically significant algaecidal activity against the toxic cyanobacterium *M. aeruginosa*. These results are similar to values obtained in some works such as that of Shi et al. 2020, where they analyzed the effects of ten plant species on the growth of *Microcystis flos-aquae*, reporting that all species showed significant inhibitory effects, with *Alternanthera philoxeroides* showing the highest degree of inhibition (77.30%), after 4 days of exposure to a concentration of 3 g/L. In another study published by Tazart et al., 2018, they observed that, aqueous extracts of *Ranunculus aquatilis* and *Nasturtium officinale* showed inhibitions of 100 and 75% in *M. aeruginosa* positively correlating the polyphenols, flavonoids and total tannins levels and the degree of inhibition of these species, similar to what was observed in our research with extracts of *A. mexicana*, *B. spectabilis* and *L. tridentata*. For their part, Ye et al., 2014, found that extracts of four Chinese herbs *Phellodendri chinensis*, *Artemisia annua*, *Scutellaria baicalensis* and *Citrus reticulata* showed strong algaecidal effects when found coexisting with *M. aeruginosa*, even showing synergistic effects when binary mixtures of the aqueous extracts were made.

The EC₅₀ values can give an idea of the degree of efficacy of an extract on a certain species of cyanobacteria and can vary significantly depending on factors such as the plant species and its geographical origin, the plant part used and its age, the solvent used in the extraction, the concentration of the extract, the exposure time and the target cyanobacteria species, among others. In our research, no significant difference was observed between the EC₅₀ values of *A. mexicana*, *B. spectabilis* and *L. tridentata*, similar to what was found by Zhang et al., 2015 (Table 3). However, in the study by Ye et al., 2014, a significant difference was observed between *Artemisia annua* extract and *Phellodendri chinensis* and *Scutellaria baicalensis* extracts. In another investigation, the efficacy of ethyl acetate extract of *Conyza canadensis* was far superior to the ethanolic extract of *Artemisia annua* (Ni et al., 2011).

Table 3 EC₅₀ value of different plant extracts on *M. aeruginosa*

Plant	Solvent	CE ₅₀ (mg/L)	Reference
<i>A. mexicana</i>	Methanol	24.47	In our study
<i>B. spectabilis</i>	Methanol	20.27	
<i>L. tridentata</i>	Methanol	13.63	
<i>Oenanthe javanica</i>	Ethanol	9.23	Zhang et al., 2015
<i>Acorus calamus</i>	Hexane	13.59	Zhang et al., 2015
<i>Phellodendri chinensis</i>	Water	0.87	Ye et al., 2014
<i>Scutellaria baicalensis</i>	Water	0.88	Ye et al., 2014
<i>Artemisia annua</i>	Water	11.16	Ye et al., 2014
<i>Citrus reticulate</i>	Water	5.27	Ye et al., 2014
<i>Conyza canadensis</i>	Ethyl acetate	31.5	Ni et al., 2011
<i>Conyza canadensis</i>	Petroleum ether	111.5	Ni et al., 2011
<i>Conyza canadensis</i>	Ethanol	250	Ni et al., 2011
<i>Erigeron annuus</i>	Ethyl acetate	24.3	Ni et al., 2011
<i>Erigeron annuus</i>	Petroleum ether	200	Ni et al., 2011
<i>Erigeron annuus</i>	Ethanol	95	Ni et al., 2011
<i>Artemisia annua</i>	Ethyl acetate	121	Ni et al., 2011
<i>Artemisia annua</i>	Petroleum ether	143	Ni et al., 2011
<i>Artemisia annua</i>	Ethanol	275	Ni et al., 2011
<i>Salvia miltiorrhiza</i>	Ethyl acetate	98.9	Yi et al., 2012
<i>Acorus tatarinowii</i>	Chlorophorm	102.5	Yi et al., 2012
<i>Salvia miltiorrhiza</i>	Chlorophorm	111.5	Yi et al., 2012
<i>Polygonum cuspidatum</i>	Chlorophorm	122.9	Yi et al., 2012
<i>Acorus tatarinowii</i>	Petroleum ether	130	Yi et al., 2012
<i>Phellodendron amurense</i>	Petroleum ether	157.8	Yi et al., 2012

Chl-a is an essential photosynthetic pigment that serves for the uptake of light energy and its subsequent transformation to biochemical energy, therefore, the concentration of Chl-a is intimately related to the growth and photosynthetic activity of cyanobacteria (Chen et al., 2019). When *M. aeruginosa* cells were exposed to varying concentrations of *L. tridentata* extract, their Chl-a content decreased significantly, being consistent with the decrease in cell density, so we speculate that one of the mechanisms of action

of this species on *M. aeruginosa* is the destruction of the photosynthetic system. Similar degradation was observed by Chen et al., 2019, when analyzing the flavonoids quercetin and luteolin isolated from pomegranate peel. In another report, Mowe et al., 2019, observed that the emergent macrophytes *Ludwigia adscendens* and *Persicaria barbata* significantly decreased Chl-a concentrations in a phytoplankton community dominated by different species of cyanobacteria and that increasing the density of *L. adscendens* and *P. barbata* resulted in a shift from cyanobacteria-dominated communities to green algae-dominated communities.

MDA is one of the most important products of membrane lipid peroxidation, so it can indirectly inform the degree of oxidative damage to the cyanobacterial membrane system induced by reactive oxygen species (ROS) (Chen et al, 2019; Zhu et al., 2021). In our study, *L. tridentata* extract dramatically increased the MDA content in *M. aeruginosa*. This suggests that the allelochemicals present in *L. tridentata* extract severely damaged the membrane integrity of *M. aeruginosa* leading to cell death. Our findings are in agreement with those reported by Li et al., 2016, when they observed that the aqueous extract of *Sagittaria trifolia* caused severe damage to the structure of *M. aeruginosa* cell membranes, increasing the MDA content 2.7-fold compared to the control. On the other hand, Zhang et al. (2011), found that aqueous extract of *Thalia dealbata* roots increased MDA content in *M. aeruginosa* up to 5.1-fold compared to the control. In another study, Meng et al. (2015), reported that MDA content in *M. aeruginosa* about 2.3-fold due to exposure to ethanolic extract of *Ailanthus altissima* for 5 days. In addition, it significantly decreased the Chl-a content, proteins and superoxide dismutase (SOD) activity.

A consequence of lipid peroxidation is the leakage of intracellular material, such as proteins, DNA and RNA, among others (Zhang et al., 2012). In the present investigation, we observed a substantial increase in the protein and nucleotide concentration of *M. aeruginosa* due to exposure to *L. tridentata* extract, inducing gradual increases in OD₂₈₀ and OD₂₆₀ values. These findings allow us to propose that *L. tridentata* extract irreversibly damages the cell membrane of *M. aeruginosa*. Our results are similar to those described by Zhang et al., 2012, reporting the extract of *Hydrilla verticillata* caused an increase in protein and nucleotide concentration in *Anabaena flos-aquae*, being almost double compared to the control. In another study published by Zhang et al., 2010, they observed that the phenolic acids p-coumaric acid and vanillic acid caused the release of nucleic acids in *M. aeruginosa*, approximately 1.6 times more compared to the control.

Conclusions

This is the first study to test the algaecidal effects in solid and liquid media of four plant species commonly used in Mexican traditional medicine. *A. mexicana* and *B. spectabilis* species showed significant effects on the growth of *M. aeruginosa*. However, *L. tridentata* presented the highest degree of effectiveness, due to its high polyphenol content. There is a positive correlation between the decrease in Chl-a content and the increase in the MDA content, proteins and nucleotides, so it is very likely that lipid peroxidation and damage to photosynthetic activity are among the main mechanisms of action of this species against *M. aeruginosa*. Because of the above, these species may be a source of effective

algaecidal compounds to mitigate toxic cyanobacterial blooms. Future studies should be conducted to identify and quantify the allelochemicals present in extracts of *L. tridentata*, *B. spectabilis* and *A. mexicana* and study their effects on *M. aeruginosa* and other toxic cyanobacterial species.

Declarations

Acknowledgements: The authors acknowledge PhD Pedro Ramírez García for the contribution of the *M. aeruginosa* species and for the use of the Bacteriology Laboratory facilities. To PhD David Chicalote Castillo for his support in the cultivation of *M. aeruginosa* and the inhibition tests in solid medium.

Ethics approval and consent to participate: Not applicable.

Consent to publish: Not applicable.

Availability of data and materials: The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Conflict of interest: The authors declare that they have no competing interests.

Funding: This research was supported by the Graduate Sub-coordination of Mexican Institute of Water Technology. The Graduate Sub-coordination did not participate in this research.

Authors' contributions: RSJ participated in the conception and design of the study, carried out the preparation of the material, the quantification of total polyphenols, the algaecide tests in solid and liquid medium, the collection and analysis of data and made the first draft of the manuscript. SEGH contributed to the conception and design of the study, purchased the reagents and materials, provided the UV-Vis equipment, arranged the loan of the cyanobacterium *M. aeruginosa*, and provided comments on the draft. AMSV participated in the conception and design of the study, provided the methodologies for the culture of *M. aeruginosa* and for the quantification of Chl-a, MDA, proteins and nucleotides, helped the statistical analysis of the results and made comments on the first draft. MLV participated in the conception and design of the study, provided the freeze-drying and deep-freezing equipment, participated in the search and selection of the plant species and their identification, and provided comments on the first draft. JRVI contributed to the conception and design of the study, participated in the quantification of total polyphenols and in the analysis of the results and the discussion. All authors read and approved the final manuscript.

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Figures

Figure 1

Total polyphenolic compounds in ethanolic and methanolic extracts of plant species

Figure 2

Antiangal activity of the extracts against *M. aeruginosa* measured as the diameter of the zone of inhibition

Figure 3

Effects of methanolic extracts on the cell density of *M. aeruginosa*

Figure 4

Inhibitory rates of methanolic extracts on *M. aeruginosa*

Figure 5

Effects of *L. tridentata* extract on the content of Chl-a, MDA, nucleotides and proteins in *M. aeruginosa*