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RESEARCH ARTICLE

Aquatic macrophytes as a source of antifouling non-toxic against bacterial biofilms and golden mussel attachment: a possible role of quorum-sensing interference

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Abstract

Biofouling in freshwater and marine environments developed on man-made aquatic surfaces causes significant economic losses. Still, this problem is magnified when it comes to invasive species, such as the golden mussel. One of the alternatives to combat it is the use of antifouling solutions; however, the vast majority focus on solutions for the marine environment. In this same context, natural antifouling solutions from macrophytes have been reported as promising to combat estuarine biofouling; however, trials with freshwater organisms are still incipient. Thus, this study evaluated the performance of 25 macrophyte extracts in inhibiting the formation and/or eradication of bacterial biofilms, settlement of the golden mussel (*Limnoperna fortunei*), as well as its toxicity effect on three different non-target model organisms of three trophic levels. Among the 25 extracts, nine demonstrated ≥ 60% inhibition of biofilm formation, with only the extracts *of Typha domingensis* and *Eichhornia crassipes* having a biofilm inhibitory effect of ≥ 70% for bacterial isolates and ≥ 60% for multispecies biofilms. Planktonic growth had distinct responses, ranging from induction, inhibition, and no effect on growth. The *T. domingensis* extract showed quorum sensing inhibition (QSI) with a dose-dependent relationship, while the *E. crassipes* extract showed QSI only at a dilution of 1.2%. These same extracts prevented the golden mussel from attaching and showed safe concentrations of 35.35% for *Pseudopediastrum boryanum* and *Daphnia magna* and 70.71% for *Pimephales promelas*. This study highlights the biotechnological potential of macrophyte extracts as a sustainable and environmentally harmless alternative for the control of micro and macrofouling in freshwater environments.

Keywords Antibiofilm · Anti-attachment · Aquatic plants · Freshwater antifouling · Quorum sensing inhibition · Natural compounds

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Introduction

Biofouling is the accumulation of biological deposits on practically all submerged surfaces in marine and freshwater environments that occurs due to the activity of a diverse community because of a complex ecological succession (Martín-Rodríguez et al. 2015). A crucial step in this succession is the initial colonization by bacteria (microfouling), which forms a biofilm associated with extrapolymeric substances and influences the attachment of other fouling organisms (*e.g.*, algae and invertebrates) (Peng et al. 2020; Ma et al. 2023). Biofouling is considered a serious economic problem for man-made aquatic structures (*e.g.*, platforms, ducts, and piers), particularly when it involves the attachment of mussels and barnacles (i.e., macrofouling) (Dobretsov and Rittschof 2020). The complexity of this problem is further magnified when associated with invasive species, like the golden mussel.

The golden mussel *Limnoperna fortunei* (Dunker, 1857) is one of the most studied invasive mussel species with a plethora of studies searching for control strategies (Boltovskoy and Correa 2015; Pereira et al. 2022). Native to mainland Southeast Asia, *L. fortunei* was accidentally introduced from the ballast water of transoceanic ships into South America in the 1990s, where it quickly invaded continental water bodies (Darrigran et al. 2020). Perhaps more importantly, the colonization of this invasive mussel into aquatic ecosystems leads to severe ecological impacts, such as competition with native species for food and space (for the consumption of phyto and zooplankton), increased cyanobacteria proliferation, and the introduction of new fish parasites, therefore causing changes in local chains and aquatic habitat structures (Cataldo et al. 2012; Boltovskoy and Correa 2015). The economic impacts are mostly related to the obstruction of hydroelectric power plants as mussels colonize hydraulic sensors and clog pipes, turbine cooling systems, chambers, and other submerged structures (Brugnoli et al. 2005; Brugnolli et al. 2011). They are also implicated in wear and changes in the conformation of hydroelectric and nuclear plant structures, water treatment plants, refineries, steel mills, and aquaculture and forestry agro-industrial systems (Boltovskoy and Correa 2015; Maranhão and Stori 2019; Fabián et al. 2021).

Over the years, several biological, physical, and chemical strategies have been attempted to control invasive mussels (Pereira et al. 2022). The most common method is the use of antifouling paints to combat biofouling. This method is well applied; however, currently, even third-generation biocides added to antifouling paint formulations have negative impacts on the aquatic environment due to their toxicity on non-target organisms (*e.g.*, inhibition of photosynthesis in microalgae, mortality in planktonic crustaceans, reduced growth of fish) (Martins et al. 2018; Mansano et al. 2018; Campos et al. 2022; Perina et al. 2023). An alternative approach that has been investigated is the use of natural products (Pereira et al. 2022) that are ecologically safer and less harmful to the environment (Agostini et al. 2021b; Pérez et al. 2021; Hamidi et al. 2022) due to their greater biodegradability and potentially lower toxicity against non-target organisms (Pérez et al. 2021).

Plant compounds have long been used to combat numerous diseases due to their antioxidant (Unuofin and Lebelo 2020), antimicrobial (Chassagne et al. 2021), anti-inflammatory (Nunes et al. 2020), anti-cancer (Khan et al. 2019), and antifouling properties (Agostini et al. 2021b). Furthermore, the use of plant compounds stands out due to the large number of chemicals present in different plant organs (Hamidi et al. 2022). Many of these compounds act as allelochemicals for their capacity to interfere with the settlement, growth, and/or development of several organisms (Hamidi et al. 2022).

The antifouling activity of plants is related to the presence of allelochemicals from the class of alkaloids, flavonoids, and tannins (Hamidi et al. 2022), which are used as additives in antifouling formulations (Hamidi et al. 2022; Neves et al. 2024). However, studies related to this topic are mainly focused on the activity of terrestrial plants (Agostini et al. 2021b; Hamidi et al. 2022), and there are still few studied with aquatic macrophytes, with only the study by Morales et al. (2024) being observed. In their study, Morales et al. (2024) observed only the antifouling effect of aqueous macrophyte extracts on the growth and formation of estuarine bacterial biofilm, and there are no reports in the literature for the effect of these plants on freshwater biofouling, especially the golden mussel, a highly invasive species with an emerging concern for its control.

Still in the literature, these plants are reported to have alkaloids, phenolic compounds, tannins, and flavonoids (Shanab et al. 2010; Silva et al. 2010), which have an influence on bacterial growth in lakes; however, their antifouling purpose in freshwater environments for the formation of bacterial biofilm and inhibition of the attachment of the golden mussel have not yet been investigated. Besides, for the development of new antifouling alternatives, current studies seek that the antifouling effects on bacterial biofilm are without affecting bacterial growth (Neves et al. 2024) but rather have an inhibitory action on quorum sensing (QS), a genetic signaling process that regulates bacterial establishment (Martín-Rodríguez et al. 2015), thus reducing bacterial resistance (Agostini et al. 2019) and making it a better alternative to inhibit biofilm.

Thereby, to assist in the development of a freshwater antifouling alternative, the present study evaluated the potential of macrophyte extracts to inhibit the formation of bacterial biofilms (microfouling) and golden mussel (*L. fortunei*) attachment (macrofouling). To assess the toxicity of macrophyte extracts more accurately, three different trophic levels were used (Pane et al. 2008): a microalga (*Pseudopediastrum*

boryanum), a crustacean (*Daphnia magna*), and a fish (*Pimephales promelas*). These species were used because they are bioindicators and have well-established toxicity tests standardized by international organizations and agencies, such as the Organization for Economic Co-operation and Development (OECD), the United States Environmental Protection Agency (USEPA), and the Brazilian Association of Technical Standards (ABNT). These tests are useful because they help to establish limits of chemical substances in the aquatic environment (Cetesb 2008), as well as evaluate new chemicals.

Materials and methods

Preparation of plant extracts

Eleven macrophytes were collected in southern Brazil (32°09′23.3″ S 52°05′57.6″ W) during the austral winter of 2020 and summer of 2021. Species identification was performed through morphological analysis of reproductive and vegetative structures using identification keys (Pott and Pott 2000; Souza and Lorenzi 2012). The preparation of plant extracts followed Morales et al. (2024). In total, 25 aqueous extracts were obtained from different organs of the macrophytes (Table 1). The separation into different plant organs of the plant occurred due to the chemical compounds being distributed qualitatively and quantitatively according to the plant organ (Cardoso et al. 2019; Ramos et al. 2022). Materials collected during both summer and winter were combined to ensure representative samples and reduce seasonal variability of the chemical compounds present in the extracts (Ramos et al. 2022). A 6 g sample of dry plant biomass (oven-dried at 60 °C until constant mass) was added to 300 mL of sterile (filtered −0.22 µm and autoclaved) natural water without salinity (0 salinity) (Agostini et al. 2019). The preparation resulted in a stock solution, considered 100%, that was diluted to 5, 10, 20, and 40%. The control treatment was sterile natural water (0% and 0 salinity).

Bacterial isolates

Bacterial isolates were obtained according to Morales et al. (2024), from biofilm-forming bacteria on acrylic, marine plywood, carbon steel (ASTM-36), and concrete substrates.

Table 1 List of plant extracts tested in bioassays

Group	Family	Species and author	Plant organ
Angiosperms	Cabombaceae	*Cabomba caroliniana* A. Gray	Leaf Stalk
Angiosperms	Cyperaceae	*Schoenoplectus californicus* (C. A. Mey.) Soják	Stalk Influorescence
Angiosperms	Menyanthaceae	*Nymphoides humboldtiana* (Kunth) Kuntze	Leaf Stalk Flower
Angiosperms	Onagraceae	*Ludwigia hexapetala* (Hook. & Arn.) Zardini et al	Leaf Stalk
Angiosperms	Onagraceae	*Ludwigia multinervia* (Hook. & Arn.) Ramamoorthy	Leaf Stalk
Angiosperms	Pontederiaceae	*Eichhornia azurea* (Sw.) Kunth	Leaf Stalk
Angiosperms	Pontederiaceae	*Eichhornia crassipes* (Mart.) Solms	Leaf Stalk Root Flower
Angiosperms	Potamogetonaceae	*Stuckenia pectinata* (L.) Börner	Mixed
Ferns and lycophytes	Salvinaceae	*Salvinia minima* Baker	Leaf Rhizoid
Ferns and lycophytes	Salvinaceae	*Salvinia herzogii* De la Sota	Leaf Rhizoid
Angiosperms	Typhaceae	*Typha domingensis* Pers	Aerial part upper Aerial part lower Inflorescence

The water samples were collected from a lagoon (32°04′56.9″ S 52°14′05.2″ W) in August 2021, when the temperature was 18 °C, and salinity zero. All isolates were screened to confirm their ability to form a biofilm (O'Toole 2011). Acquisition and identification of the bacterial isolates by sequencing were performed as described in Morales et al. (2024). Sequences were submitted to the Genbank (accession number SUB 13564963 (OR000433-OR000442)). Isolates were added to the *Microfouling Bank* of the Laboratory of Organic Microcontaminants and Aquatic Ecotoxicology—CONECO, Institute of Oceanography – IO at the Federal University of Rio Grande (FURG).

Isolated bacteria were used to carry out the biofilm inhibition and eradication assays, as well as the potential growth inhibition of planktonic bacteria. Taxonomic information of all bacterial isolates can be seen in Supplementary Table S1.

Bacterial community

Water samples were collected from a lagoon (32°09′44.6″ S 52°06′04.4″ W) at a temperature of 18 °C to obtain the multispecies bacterial community inoculate for antibiofilm assays. Water samples were filtered (7 µm) to remove phytoplankton and zooplankton organisms (Agostini et al. 2021a).

Bacterial density assays

Each bacterial isolate and multispecies bacterial community sample was used separately to perform bacterial density assays (Fig. 1). These were carried out in 96 acrylic multi-well plates (Citotest Labware Manufacturing Co. Ltd, Jiangsu, China) with eight replicates per treatment. A suspension of bacterial inoculum in nutrient broth (K25-1216, Laboratories Conda S.A., Madrid, Spain) with an initial density of 10^6 bacteria mL^{-1} was used (Agostini et al. 2019). The nutrient broth was chosen to avoid misinterpretation and not to mask the results, eliminating a confounding factor from the results.

To test the inhibition of biofilm formation (IBF), an initial screening assay containing 100 µL of plant extracts at increasing dilutions (0, 5, 10, 20, and 40%) was mixed with the bacterial inoculum and incubated in the dark at 25 °C for 48 h (Agostini et al. 2019). Measurements of the biofilm density for the IBF were performed by removing the supernatant, drying the biofilm (60 °C for 1 h), staining

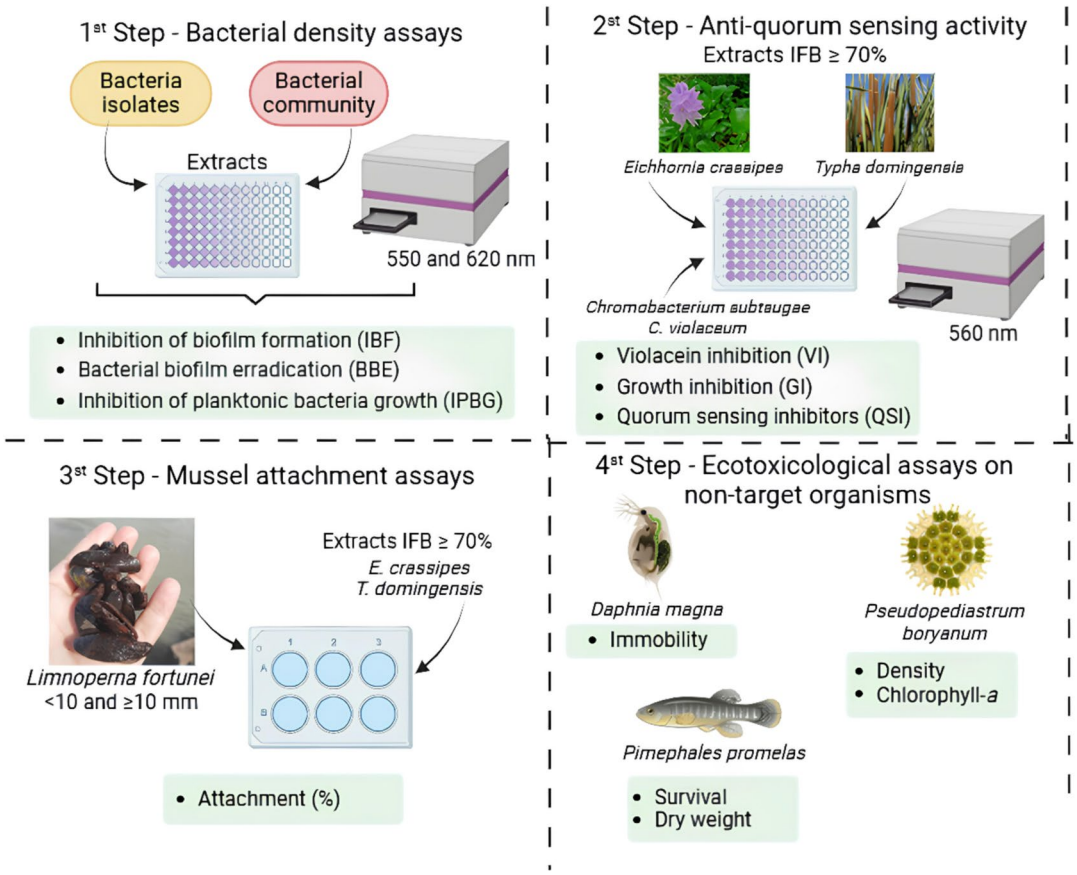

Fig. 1 Scheme of procedures adopted to evaluate the antifouling effect of aqueous extracts of macrophytes

with violet crystal (0.4%) for 15 min, discarding the violet crystal solution and rinsing the wells three times with sterile Milli-Q water, and solubilizing with ethanol (Abs.) for 30 min. The optical density was measured on a microplate reader (Filtermax F5, Molecular Devices, San Jose, USA) at 550 nm (O'Toole 2011; Agostini et al. 2019).

Plant extracts producing IBF ≥ 60% were then used to carry out the bacterial biofilm eradication (BBE) and inhibition of planktonic bacteria growth (IPBG) assays. For the BBE assays, biofilms were established from 200 µL of bacterial inoculum incubated in the dark for 24 h. Subsequently, the biofilm was exposed to 200 µL of the test solutions (0, 5, 10, 20, and 40%) for 48 h in the dark at 25 °C (Morales et al. 2024). Biofilm density for BBE was analyzed in the same way as for IPBG.

The IPBG assay was performed to ascertain whether the biofilm inhibition activity was due to antibiofilm (no effect on growth and single effect on the biofilm) or antibiotic activity (growth reduction) (Agostini et al. 2019). For the IPBG assay, the growth inhibition of planktonic bacteria was used as the endpoint, thus estimating the difference in optical density (620 nm) at the beginning and end of the incubation period (48 h in the dark at 25 °C) (Agostini et al. 2019; Do Vale et al. 2021). Controls for the IBF, BBE, or IPBG assays were considered 100%. Results were, therefore, expressed as a percentage of inhibition (IBF or IPBG) or eradication (BBE) compared to the control.

Anti-quorum sensing activity: violacein inhibition

To confirm the antibiofilm effect of the extracts, the violacein inhibition assay was used to quantify the quorum sensing inhibition (QSI) activities (Fig. 1) of the same aqueous macrophyte extracts used in the toxicological assays (*E. crassipes* and *T. domingensis*) (described in the "Ecotoxicological assays on non-target organisms" section), against two Gram-negative biosensor microorganisms: *Chromobacterium subtsugae* CV017 to detect short chain (C4-C6) acyl-homoserine lactone (AHL) inhibition (Chernin et al. 1998) and *C. violaceum* ATCC 12472 to detect long chain (C10 +) AHL inhibition (Morohoshi et al. 2008). Extracts were resuspended in sterile, deionized water to a stock of 20 mg.mL^{-1}, considered 100%. One hundred microliters of an overnight *C. subtsugae* CV017 and *C. violaceum* ATCC 12472 culture, grown in 5 mL Luria–Bertani (LB) broth, was used to inoculate test tubes containing 3 mL LB broth, which were exposed to varying dilutions (0.07, 0.15, 0.30, 0.60, 1.20%) of respective extracts and incubated at 30 °C for 18 h with agitation (150 rpm) in a rotary suspension mixer (SM-3600–0018, Lab YIHDER Technology CO, Taiwan) (Chenia 2013). These dilutions were used because they presented antibiofilm and anti-attachment effects in the previous stages and did not present a toxicological effect on non-target organisms. The growth control was LB broth inoculated with only *C. subtsugae* CV017 and *C. violaceum* ATCC 12472 culture (no extract). Vanillin (Sigma-Aldrich) was used as QSI-positive control and tested at same dilution range as extracts.

Following incubation, culture growth readings were obtained with a Glomax Multi + Detection System microtiter plate reader at OD_{600} nm (Promega). One mL of the cultures was subjected to centrifugation at 13,000 rpm for 10 min (Labnet Prism microcentrifuge), thus precipitating the insoluble violacein. Culture supernatants were discarded, and pellets were resuspended in 1 mL of dimethyl sulfoxide (DMSO) (Chenia 2013). Following centrifugation at 13,000 rpm for 10 min, cells were pelleted, and the violacein-containing solutions were quantified at OD_{560} nm using the Glomax Multi + Detection System microtiter plate reader (Chenia 2013). Violacein inhibition was assessed in triplicate on two separate occasions. The percentage of violacein inhibition was calculated according to Chenia (2013). If extracts demonstrated violacein inhibition (%VI) and growth inhibition (%GI) of ≥ 50% and < 40%, respectively, they were considered good quorum sensing (QS) inhibitors. However, a %VI and %GI of ≥ 50% and ≥ 40%, respectively, were considered bactericidal activity rather than QSI (Rambaran et al. 2024).

Mussel attachment assays

The extracts that showed an inhibitory effect ≥ 70% in the IBF (single and multispecies bacteria) assays were used to evaluate the ability to inhibit golden mussel attachment (Fig. 1). Specimens of *L. fortunei* were collected from the channel walls of the first elevation of the Rio Grande Sanitation Company (CORSAN) (32°3′14.39″ S 52°22′18.28″ W) and transported in plastic containers without water to the laboratory. In the laboratory, the mussels were separated with scissors, cleaned with dechlorinated water, and transferred to an 80 L black plastic tank containing dechlorinated water circulating through a biological filter at a controlled temperature of 20 °C and photoperiod 12L:12D. Mussels were fed twice a day with a commercial concentrate of *Chlorella vulgaris* (Beyerinck (Beijerinck), 1890) (ChloFresh, Algasul, Rio Grande, Brazil) at 10^9 cells mL^{-1}. Mussels were kept acclimatized under these conditions for 2 weeks.

Two separate assays were performed according to mussel size: < 10 mm and ≥ 10 mm (Cataldo et al. 2005). Mussels were transferred from the maintenance tank to a transparent plastic container (5 L) to verify their substrate exploration behavior (Longo et al. 2021). Only individuals showing such behavior were selected for the attachment assays. Treatments were composed of 0, 5, 10, 20, and 40% of the test solution of each selected plant extract, with 30 individuals per

treatment. Assays were carried out in 6-well plates (Barloworld Scientific Ltd., Stone, UK), each well containing one mussel and 10 mL of the test solution (Longo et al. 2021). Exposure time was 72 h at 20 °C in the dark (Longo et al. 2021). At the end of the exposure, mussels adhered to the well walls were counted, and results were expressed as a percentage of attachment.

Ecotoxicological assays on non-target organisms

As all the different organs of the plants were successful in inhibiting the attachment of the golden mussel, mixtures of parts of *E. crassipes* and *T. domingensis* were used for ecotoxicological assays (Fig. 1). Extracts were freeze-dried and then used to prepare solutions with dilutions of 0, 6.25, 12.50, 25, 50, and 100% of each extract. Three freshwater non-target model organisms were selected: the microalga *Pseudopediastrum boryanum* [(Turpin) E.Hegewald 2005] (Chlorophyta, Hydrodictyaceae), the crustacean *Daphnia magna* (Straus, 1820) (Arthropoda, Daphniidae), and the fish *Pimephales promelas* (Chordata, Cyprinidae) (Rafinesque, 1820).

Microalgae assay: density and chlorophyll-a

The assay with *P. boryanum* was done following OECD guidelines 201 (2011), with some modifications, such as the use of a WC culture medium (Guillard and Lorenzen 1972) to enrich the test solutions. The assay was carried out in 50 mL Erlenmeyer flasks containing 49 mL of the test solution and 1 mL of microalgae inoculum at a density of 10^4 cells mL^{-1} at 24 °C, continuous lighting of 7000 lx, and constant agitation on a shaking table. The duration of the test was 72 h (±2 h), with an initial pH of 6.6–7.0. Each treatment had four replicates. At the beginning of the test (0 h) and at the end (72 h), an aliquot of 1 mL was taken, and 200 µL of formaldehyde was added to fix the algal culture (final concentration 0.4%). The endpoint analyzed was cell concentration (cells mL^{-1}) for each treatment, estimated as the difference in cell count between 72 and 0 h with a Neubauer counting chamber. The control treatment used uninoculated WC culture medium.

After 72 h, 5 mL aliquots were removed and centrifuged (4000 rpm) for 10 min. The supernatant was discarded, and 3 mL of methanol (99.9%) was added. The samples were kept under refrigeration (5 °C) for 12 h in the dark. The samples were then centrifuged (4000 rpm), and the optical density of the supernatant was determined using a UV–VIS spectrophotometer (UV mini-1240, Shimadzu, Kyoto, Japan) at 663 and 750 nm. Chlorophyll-*a* (µg L^{-1}) extraction and concentration estimation were performed according to Mackinney (1941).

Cladoceran assay

The acute toxicity test with *D. magna* was carried out following ABNT NBR 12713 (2022) guidelines in 6-well plates (Barloworld Scientific Ltd., Stone, UK), with four replicates, each containing five organisms and 10 mL of the test solution (test solution ratio of 2 mL $organism^{-1}$). Plates were incubated for 48 h (±1 h) at a temperature of 20 °C (±2 °C) and a photoperiod of 12 h:12 h. The organisms were not fed during the assay. At the end of the exposure time, the effect of the extracts on the mobility of the organisms was observed, and results were expressed as immobility organisms (%) per treatment.

Fish assay: survival and dry weight

From 0 to 24 h after hatching, *P. promelas* larvae were subjected to a 7-day long static test with daily water renewal. Survival and larval dry weight were determined at the end of the test according to EPA—821 R—02—013—Method 1000.0 (2002). Each treatment had 4 replicates with 10 larvae and 250 mL of test solution placed within 500 mL containers. These were incubated at 25 °C (±1 °C), with a photoperiod of 16 h:8 h, and a light incidence of 500–1000 lx. Larvae were fed with *Artemia nauplii* at a concentration of 700 to 1000 ind mL^{-1}, three times a day. Larval survival was estimated daily. At the end of the exposure time, the dry weight of larvae was determined by drying in an oven (60 °C) for 24 h.

Statistical analysis

For toxicological responses, the values of the lowest concentration (or dilution) with observed effect (LOEC), the highest concentration (or dilution) at which no effect was observed (NOEC), and the safe concentration (or dilution) of extracts through the arithmetic mean between LOEC and NOEC were also calculated (Zagatto and Bertoletti 2008). *T*-Student tests were performed, using Bonferroni correction for multiple comparisons, to observe potential differences between treatments and respective controls in BFI, BBE, and PBGI assays for single and multispecies bacteria. One-way ANOVA was run to verify significant differences between treatments in the assays with *P. boryanum*, *P. promelas* (dry weight), and violacein inhibition (both *Chromobacterium* species biosensors). In all cases, the assumptions of normal distribution of residues (Shapiro–Wilk test) and homoscedasticity (Levene test) were verified. When alternative hypotheses with a confidence level of 95% were accepted, the Tukey post-hoc test was run. Survival of *D. magna* and *P. promelas* and mussel attachment data were analyzed with Generalized Linear Models (GLM) with binomial distribution with logit link function. Statistical analysis was conducted with the use of GraphPad Prism 8.4 (GraphPad, USA).

Results

Identification of bacterial isolates

The isolation of bacteria resulted in the identification of nine taxa (Supplementary Table S1). For each of the four substrates tested (concrete, marine plywood, acrylic, and ASTM-36 carbon steel), three species were identified (Supplementary Table S1), with *Pseudomonas* species being isolated from all the substrates. Each substrate displayed two other unique species of biofilm-forming bacteria.

Bacterial density assays

Bacterial isolates

Of the 25 different extracts initially tested, nine showed IBF ≥ 60% for at least one of the tested bacteria ($p < 0.05$) (Fig. 2): flower, leaf, stalk, and root of *Eichhornia crassipes*; leaf of *Salvinia herzogii*; inflorescence of *Schoenoplectus californicus*; stalk of *Nymphoides humboldtiana*; and inflorescence and the upper aerial part of *Typha domingensis*. Extracts from *E. crassipes* (Fig. 2A–D) and

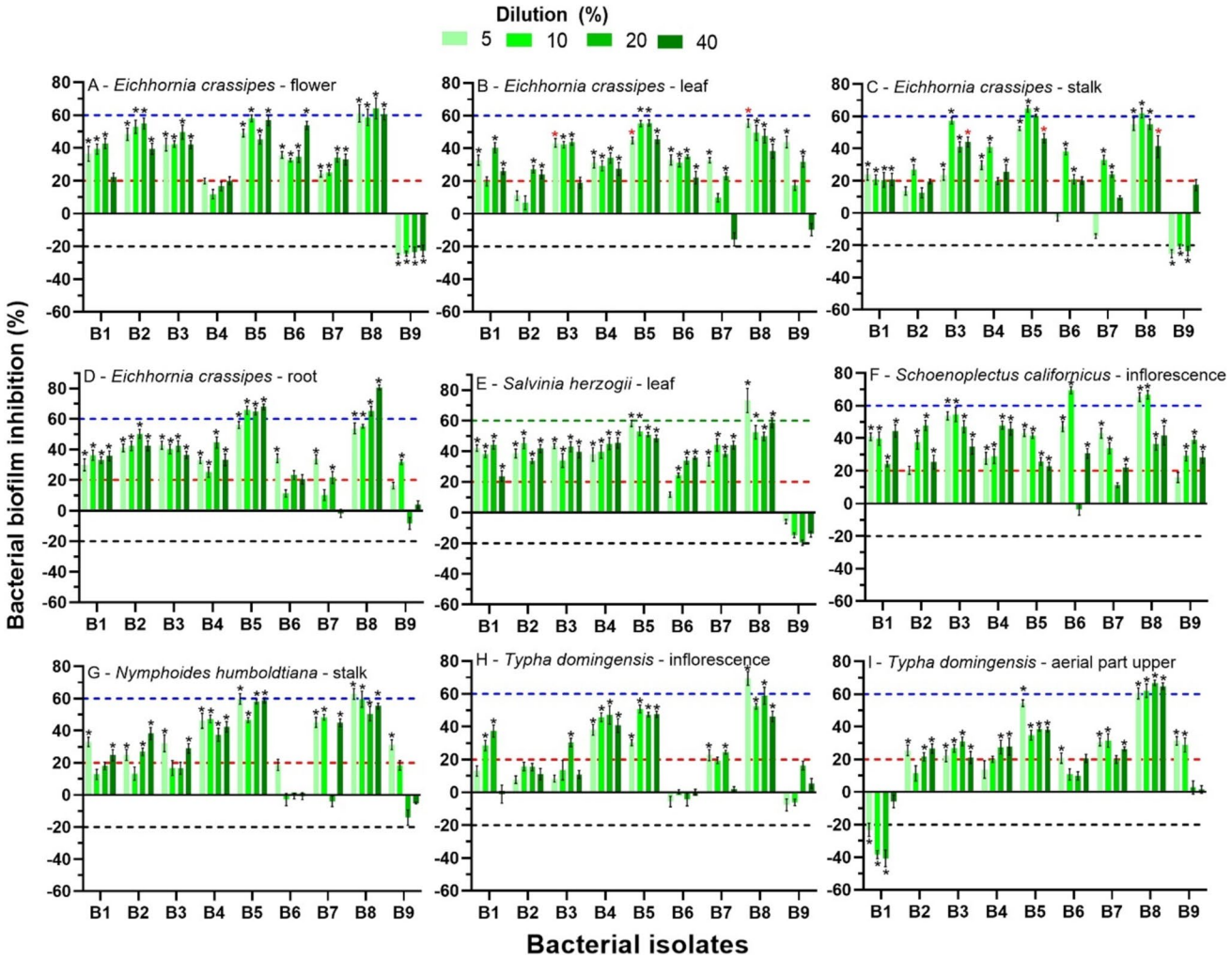

Fig. 2 Mean (±SD) inhibition of biofilm formation (IBF; %) of nine biofilm-forming bacterial isolates (B1 = *Pseudomonas* sp.; B2 = *Psychrobacillus psychrodurans*; B3 = *Citricoccus nitrophenolicus*; B4 = *Pseudomonas putida*; B5 = *Paeniglutamicibacter kerguelensis*; B6 = *Acinetobacter haemolyticus*; B7 = *Bacillus vietnamensis*; B8 = *Acinetobacter bohemicus*; B9 = *Pseudomonas rhodesiae*) following exposure to nine (**A**–**I**) selected extracts of aquatic macrophytes. *black asterisk denotes significant differences compared to the control group ($p < 0.05$). *red asterisk indicates the results of biofilm eradication assays ($p < 0.05$), combined in the same figure e. Red line = inhibition of biofilm formation; black line = biofilm formation induction activity; blue line = inhibition of biofilm formation ≥ 60% (inhibition in all cases is read as positive values)

T. domingensis (Fig. 2H, I) were the most effective, resulting in IBF > 70% for at least one dilution for each of the nine bacterial isolates ($p < 0.05$) (Fig. 2). In terms of IBF, the bacterium *Acinetobacter bohemicus* showed greater inhibition by 88.89% (8/9) of plant extracts (IBF > 60%) at least one dilution ($p < 0.05$). All data regarding biofilm inhibitory effects of extracts are summarized in Supplementary Table S2.

For the BBE assay, only extracts of leaves (5%) and stalks (40%) of *E. crassipes* (Fig. 2B, C) had an eradication effect ($p < 0.05$). For both extracts, this effect was observed only against *Citricoccus nitrophenolicus*, *Paeniglutamicibacter kerguelensis*, and *A. bohemicus*. Six of the selected nine extracts demonstrated a bactericidal effect against at least one bacterial species in some dilution ($p < 0.05$). An antibiofilm effect ($p > 0.05$) was also observed for some dilutions of *E. crassipes*, *N. humboldtiana*, and *T. domingensis* (Fig. 3). However, for most bacteria and dilutions tested, extracts had a growth-inducing effect (Fig. 3; $p > 0.05$).

Bacterial community

Biofilm formation by the multispecies bacterial community was inhibited (≥ 60%; $p < 0.05$) by nine extracts: flower, stalk, and roots of *E. crassipes*; leaves of *S. herzogii*; inflorescence of *S. californicus*; flower and stalk *N. humboldtiana*; and inflorescence and upper aerial part of *T. domingensis* (Fig. 4A). Two extracts were highly effective (inhibition > 70%), i.e., the roots of *E. crassipes* at the 40% dilution and the upper aerial part of *T. domingensis* at 20% dilution ($p < 0.05$) (Fig. 4A).

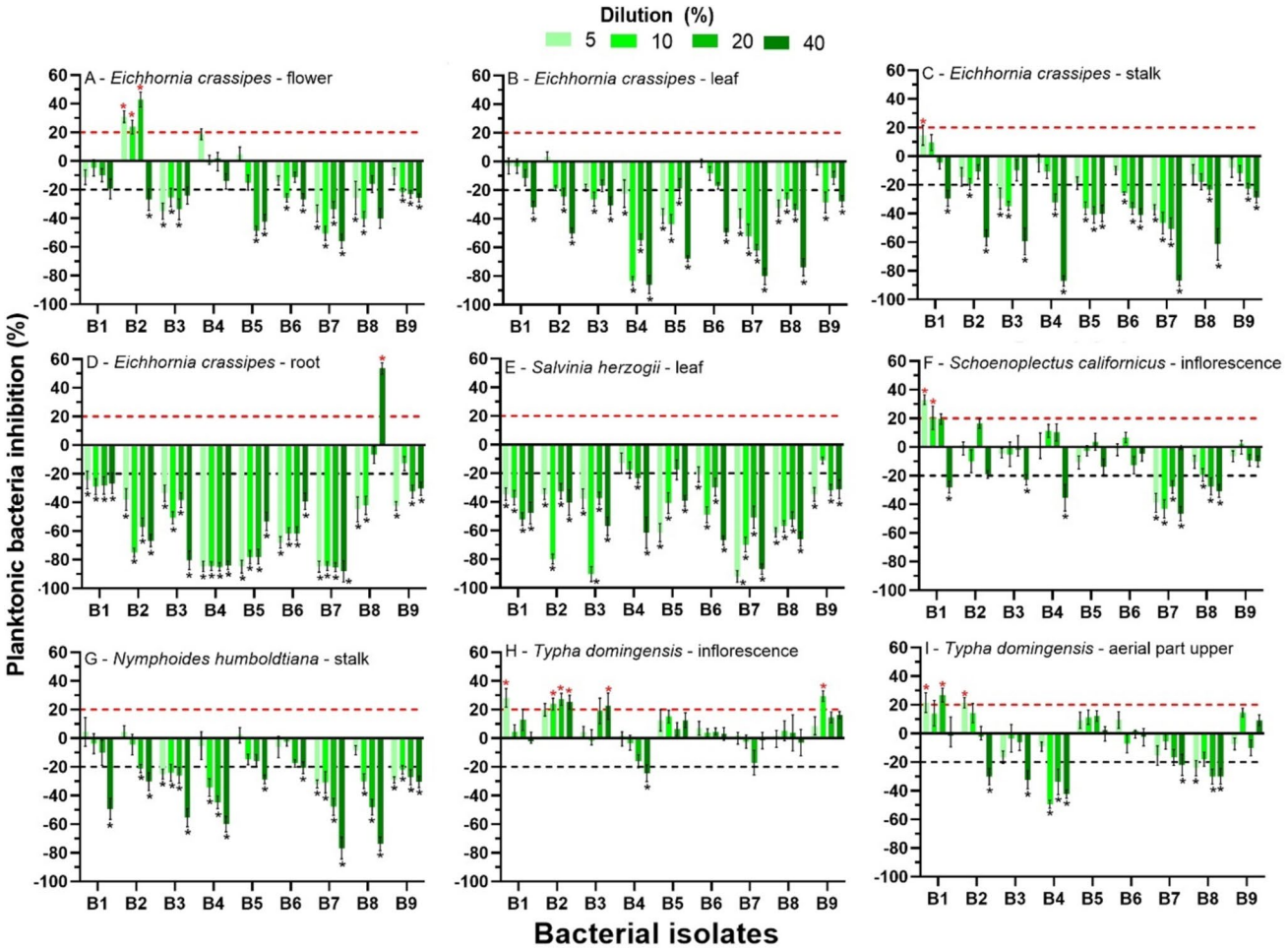

Fig. 3 Mean (±SD) response (% planktonic bacteria inhibition) of nine bacterial isolates (B1 = *Pseudomonas* sp.; B2 = *Psychrobacillus psychrodurans*; B3 = *Citricoccus nitrophenolicus*; B4 = *Pseudomonas putida*; B5 = *Paeniglutamicibacter kerguelensis*; B6 = *Acinetobacter haemolyticus*; B7 = *Bacillus vietnamensis*; B8 = *Acinetobacter bohemicus*; B9 = *Pseudomonas rhodesiae*) to the exposure to nine (**A–I**) selected extracts of aquatic macrophytes. Asterisk (*) = presents statistical difference when compared to the control group; red asterisk (*) = antibiotic effect; black asterisk (*) = inducing planktonic growth. Dilutions that showed no difference when compared to the control group = antibiofilm activity. Red line = antibiotic effect (reduced planktonic growth); black line = effect of inducing planktonic growth

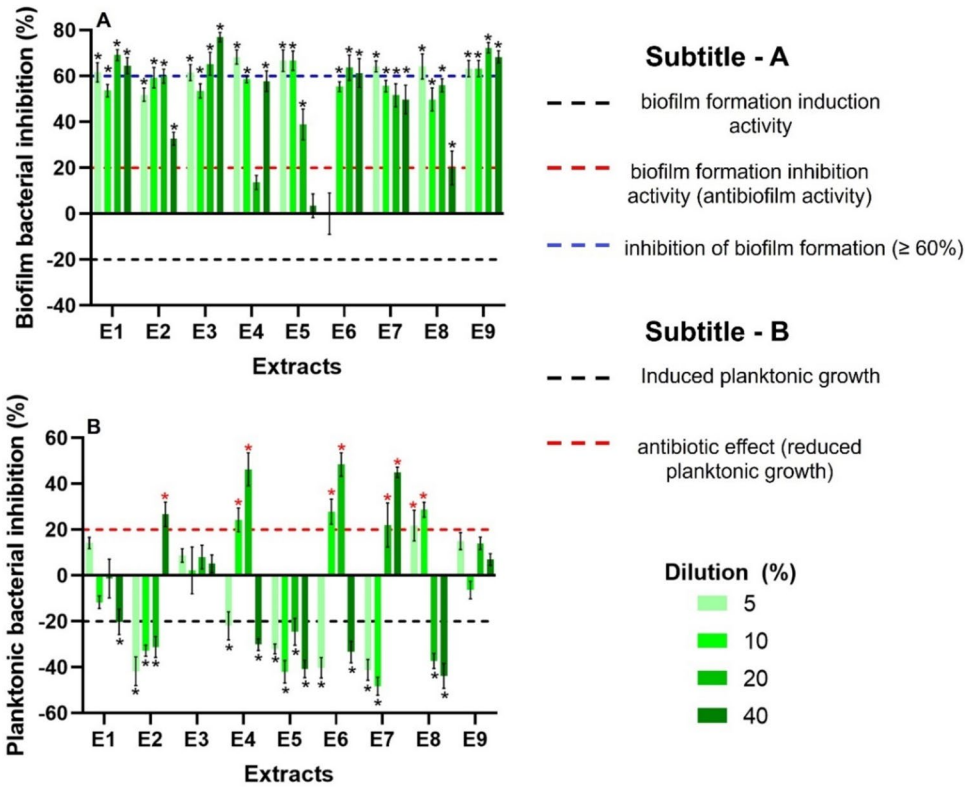

Fig. 4 Mean (± SD) response biofilm bacterial inhibition (%) (**A**) and planktonic bacterial inhibition (%) (**B**) of the bacterial community. E1 = *Eichhornia crassipes* flower; E2 = *E. crassipes* stalk; E3 = *E. crassipes* root; E4 = *Salvinia herzogii* leaf; E5 = *Schoenoplectus californicus* influorescence; E6 = *Nymphoides humboldtiana* flower; E7 = *N. humboldtiana* stalk; E8 = *Typha domingensis* influorescence; E9 = *T. domingensis* aerial part upper. Asterisk (*) = presents statistical difference when compared to the control group; red asterisk (*) = antibiotic effect

As for the planktonic growth assays, only the extracts of flowers and roots of *E. crassipes* and *T. domingensis* upper aerial part demonstrated an antibiofilm effect ($p > 0.05$) on planktonic growth (Fig. 4B). The remaining extracts varied in their response depending on their dilution in terms of induction ($p < 0.05$) or inhibition ($p < 0.05$) of planktonic growth (Fig. 4B). None of the extracts had a biofilm eradication effect on the multispecies bacterial community (Fig. 4B). None of the extracts that present satisfactory results with bacterial isolates (*E. crassipes* and *T. domingensis*) demonstrated the same ability to eradicate biofilms of the multispecies bacterial community (Fig. 4B).

Anti-quorum sensing activity

For short-chain AHL-producing *C. subtsugae* 017, QSI was observed with the two extracts tested, i.e., *E. crassipes* extract demonstrated 50–67.30% VI from 0.15 to 1.20%, while the *T. domingensis* extract demonstrated a 66.70% VI at 1.20% only (Fig. 5). The QSI positive control vanillin also demonstrated 67.82% VI at 1.20%. Extracts significantly influenced the %VI of *C. subtsugae* 017 in a dose-dependent manner ($p < 0.05$).

For the long-chain AHL-producing *C. violaceum* ATCC 12472, a 57.29% VI was obtained with the *T. domingensis* extract at 1.20%. For *E. crassipes*, no note-worthy QSI. The vanillin-positive control had a 78.30% VI at 0.60% but was bactericidal at 1.20%. Although only *T. domingensis* reached the target 50% VI, a dose-dependent relationship was also observed between extracts and *C. violaceum* ATCC 12472 (Fig. 5). All data regarding QSI and %VI are summarized in Supplementary Table S3.

Mussel attachment assays

All six extracts tested inhibited the attachment of golden mussels (Fig. 6). The proportion of successfully attached mussels (average) varied more in relation to the different dilutions ($p < 0.05$) than due to the size of the mussels ($p > 0.05$). The extract from the inflorescence of *T. domingensis* was the most effective in reducing mussel attachment (Fig. 6), with an ≥ 80% reduction in attachment ($p < 0.05$).

Ecotoxicological assays

Regarding the toxicity of *T. domingensis* extract, the 50 and 100% dilution had a significant negative effect on cell

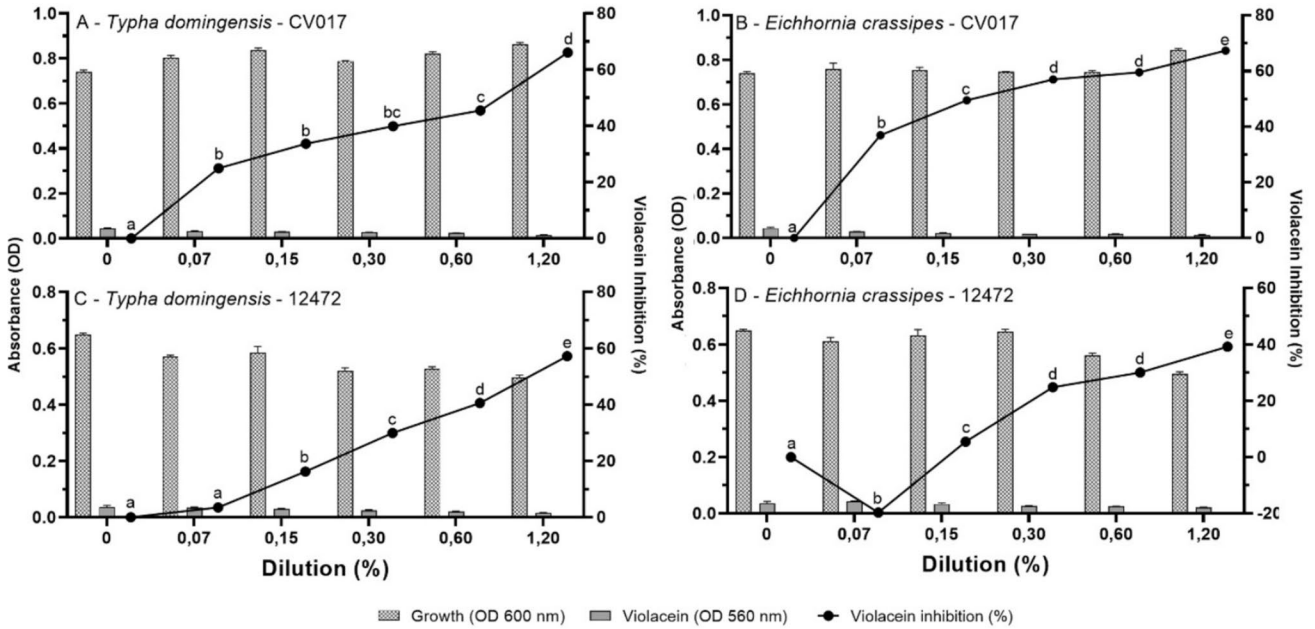

Fig. 5 Quantitative analysis of the dilution inhibitory effects (Mean (± SD)) of aqueous extracts *Typha domingensis* (**A** and **D**) and *Eichhornia crassipes* (**B** and **C**), on growth and violacein production by two biosensors *Chromobacterium subtsugae* CV017 (short chain) and *Chromobacterium violaceum* ATCC 12472 (long chain). Different letters of violacein inhibition = denote significantly different means ($p < 0.05$) between dilutions in each extract

density and chlorophyll-*a* content of *P. boryanum* ($p < 0.05$) and on the mobility of *D. magna* ($p < 0.05$) (Table 2). The survival and weight of *P. promelas* (Table 2) were only negatively affected at 100% dilution (undiluted extract; $p < 0.05$). A similar response pattern was observed for the *E. crassipes* extract in relation to the three species (Table 2). The 50 and 100% dilution had a significant negative effect on the cell density of *P. boryanum* ($p < 0.05$) and the mobility of *D. magna* ($p < 0.05$). For *P. boryanum* chlorophyll-*a*, only the 100% dilution showed negative effects ($p < 0.05$). The survival and weight of *P. promelas* (Table 2) were also only negatively affected at 100% dilution ($p < 0.05$).

For the *T. domingensis* extract, the NOEC for *D. magna* and *P. boryanum* was 25%, LOEC 50%, and a safe dilution of 35.35% was estimated for these organisms, while for *P. promelas*, the NOEC was 50%, LOEC 100%, with a safe dilution of 70.71% (Table 3). For the *E. crassipes* extract toxicity, the results were similar (Table 3). All data regarding *T. domingensis* and *E. crassipes* extracts are presented in Tables 2 and 3 and Supplementary Fig. S1.

Discussion

Several studies indicate that the eleven species of aquatic macrophytes species tested in the present study had a negative effect on the growth of algae, plants, bacteria, and bacterial biofilm (Takao et al. 2011; Chicalote-Castillo et al. 2017; Jiménez 2020; Morales et al. 2024). The results of the present study demonstrated that only extracts of five species (*E. crassipes*, *S. herzogii*, *S. californicus*, *N. humboldtiana*, and *T. domingensis*) were considered effective (> 60%) in inhibiting freshwater bacterial biofilm. The *E. crassipes* and *T. domingensis* extracts resulted in bacterial biofilm inhibition of > 70%. It is thus apparent that the inhibition potential of allelochemicals may vary depending on macrophyte species and organs, an effect also observed by Cardoso et al. (2019) and Ramos et al. (2022).

The antibiofilm potential of the macrophyte extracts was assessed against selected bacterial strains as well as naturally occurring multispecies bacterial communities. Most of the strains tested are from the phylum Proteobacteria, specifically the Gamma-Proteobacteria class. Proteobacteria are recognized for their high phylogenetic diversity and phenotypic versatility, which enables the colonization of different habitats (Kersters et al. 2006; Zinger et al. 2011). This phylum is reported to be abundant in natural waters, with many representatives that are able to grow in substrates such as lignin, calcarenites, acrylics, marine plywood, ASTM-36 cable carbon steel and sediments from aquatic environments (Agostini et al. 2021a; Gusmão et al. 2023; Morales et al. 2024), and can be isolated from biofilms on ship hulls and rocky substrates (Muthusamy et al. 2017; Bergo et al. 2021; Ferreira et al. 2022). This confirms that the species used in the present study are representative of bacterial biofilms found on different substrates in the aquatic environment.

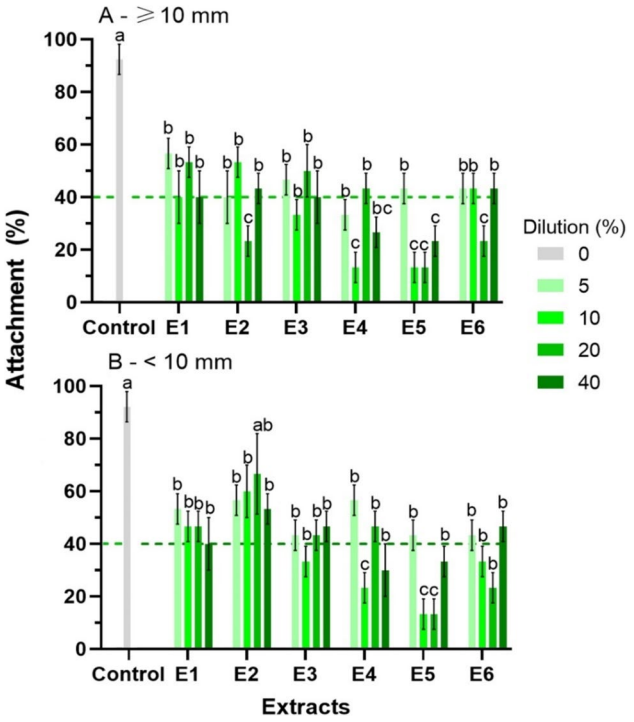

Fig. 6 Mean (± SD) attachment (%) of the golden mussel *Limnoperna fortunei* after 72 h exposure to extracts of different aquatic macrophytes (E1 = *Eichhornia crassipes* flower; E2 = *E. crassipes* leaf; E3 = *E. crassipes* stalk; E4 = *E. crassipes* root; E5 = *Typha domingensis* inflorescence; and E6 = *T. domingensis* upper aerial part) at varying dilutions (0 to 40%): **A** Assays with individuals ≥ 10 mm. **B** Assays with individuals < 10 mm. Percent in green = percentage inhibition of attachment ≥ 60% when compared to the control. Different superscript letters denote significant differences ($p < 0.05$)

Table 2 Summary of ecotoxicity results following exposure to aquatic macrophyte extracts

Extract/ Dilution (%)	*Pseudopediastrum boryanum*		*Daphnia magna*		*Pimephales promelas*
	Density	**Chlorophyll-*a***	**Immobility**	**Survival**	**Weight**
Typha domingensis					
6.25	No	No	No	No	No
12.50	No	No	No	No	No
25	No	No	No	No	No
50	Yes	Yes	Yes	No	No
100	Yes	Yes	Yes	Yes	Yes
Eichhornia crassipes					
6.25	No	No	No	No	No
12.50	No	No	No	No	No
25	No	No	No	No	No
50	Yes	Yes	Yes	No	No
100	Yes	Yes	Yes	Yes	Yes

Yes = showed toxic effect; No = showed no toxic effect. Dilutions here are read as dilutions from the 100% original extracts

The Gram-negative bacterium *A. bohemicus* was inhibited by most of the macrophyte extracts, unlike the Gram-positive bacteria *Psychrobacillus psychrodurans*, *Bacillus vietnamensis,* and *C. nitrophenolicus* which had little inhibition. Due to their external lipopolysaccharide membrane, Gram-negative bacteria have greater selectivity and consequently less sensitivity to external factors, such as exposure to natural extracts (Awolola et al. 2014; Seibert et al. 2019). In the present study, however, *A. bohemicus* did not present the expected systematic response according to its cell wall Gram reaction. The biofilm inhibition strategies are multifactorial and may be related to other mechanisms not necessarily related to cellular permeability, such as the inhibition of quorum sensing (QS) between biofilm-forming bacteria, surface modulation of bacterial adhesion, or degradation of the biofilm matrix (Srinivasan et al. 2021; Rambaran et al. 2024).

Biofilms are structured as complex colonies of microorganisms enveloped in an extracellular polymeric matrix (EPM) that provides protection against external chemical agents (Agostini et al. 2018). Along with EPM, there is an increase in the detection, production, and release of signaling molecules that help regulate biofilm formation (QS process) (Chattopadhyay et al. 2022). The QS process coordinates population behavior and regulates gene expression through bacterial cell-to-cell communication (Xiao et al. 2022). Thus, QS-inhibiting phytochemicals arouse considerable interest among researchers (Martínez et al. 2019; Mulat et al. 2019; Rambaran et al. 2024).

Based on the results of eradication, planktonic growth, and QS inhibition assays, some hypotheses can be raised and will be discussed later throughout the text. For the planktonic growth inhibition assay, three types of response were observed: antibiotic (growth reduction), inductive (growth induction), and antibiofilm (no effect on growth and single effect on the biofilm). The inductive effect was observed for all extracts tested at least one of the dilutions (5, 10, 20, and 40%) for the bacterial species and bacterial community. However, the *T. domingensis* extracts resulted in a lower growth induction when compared to the other extracts, showing a greater antibiofilm effect. The induction of bacterial growth may have occurred due to the chemical composition of the extracts and their respective dilutions, where some treatments may have more compounds to induce growth than to inhibit it. These differential effects between inhibition and induction are already reported and explained by the specificity of the chemical composition of each macrophyte species (Santonja et al. 2018). Further, they can be attributed to differences in strains due to attached bacterial biofilm, cell wall properties, and induced oxidative stress (Mulderij et al. 2005).

The extracts from macrophytes were not as effective in the biofilm eradication assays as they were at inhibiting biofilm formation. Eradication rates ≥ 60% were only

Table 3 Ecotoxicological responses to *Typha domingensis* and *Eichhornia crassipes* extracts

Macrophyte extract	Test-organism	Observed-response	LOEC (%)	NOEC (%)	Mean ± SD
Typha domingensis	*P. boryanum*	Density	50	25	35.35 ± 12.5
		Chlrophyll-*a*	50	25	35.35 ± 12.5
	D. magna	Immobility	50	25	35.35 ± 12.5
	P. promelas	Survival	100	50	70.71 ± 25
		Weight	100	50	70.71 ± 25
Eichhornia crassipes	*P. boryanum*	Density	50	25	35.35 ± 12.5
		Chlrophyll-*a*	100	50	70.71 ± 25
	D. magna	Immobility	50	25	35.35 ± 12.5
	P. promelas	Survival	100	50	70.71 ± 25
		Weight	100	50	70.71 ± 25

LOEC = observed effect dilution (lowest dilution at which effects are observed); NOEC = no observed effect dilution (highest dilution at which no effect is observed); mean = safe dilution (geometric mean between OEC and NOEC). Dilutions here are read as dilutions from the 100% original extracts

observed with *E. crassipes* leaf (5% dilution) and stalk (40% dilution) extracts against the bacteria *C. nitrophenolicus*, *Paeniglutamicibacter kerguelensis*, and *A. bohemicus*. When evaluating Caatinga plant extracts, Agostini et al. (2020) and (2019) also found that the rate of inhibition of marine biofilms was much higher than their eradication. This response is not entirely unexpected as biofilm architecture can improve the defense against external chemical agents (Agostini et al. 2018, 2019). Biofilm-forming bacteria can withstand and resist different environmental conditions, such as low nutrient availability and the action of chemical agents such as the compounds present in extracts (Davey and O'toole 2000; Srinivasan et al. 2021; Chattopadhyay et al. 2022).

The low eradication efficacy combined with the non-inhibition of planktonic growth suggests that macrophyte extracts have mechanisms other than toxicity for inhibiting biofilm formation. One possible explanation is that the presence of chemical substances in the extracts may interfere with the QS process. These results were mainly evidenced by the *T. domingensis* extract, as it had a specific inhibitory effect on biofilm formation. This is corroborated by the QSI assay results. The *T. domingensis* extract demonstrated inhibition of both short- and long-chain AHL-producing biosensor strains, suggesting broad-spectrum QSI activity. The *E. crassipes* extract, however, was only effective for short-chain AHL QSI inhibition.

Chemical substances with QSI potential are extremely relevant in the development of new antibiofilm agents, as they reduce the risk of bacterial resistance (Chenia 2013; Borges and Simões 2019). However, other mechanisms may be involved in the biofilm inhibition process, such as the reduction of enzymatic activity. Aqueous and methanolic extracts of the macrophyte *Stuckenia pectinatus* (formerly *Potamogeton pectinatus*) have been reported to reduce the alkaline phosphatase activity of bacterial communities, probably due to enzyme complexation by humic acids (They et al. 2015).

Reproductive organs and leaves are the most investigated plant parts as they usually present the most satisfactory results against biofouling (Agostini et al. 2021b). In the present study, extracts from different plant organs had a significant effect against bacterial biofilm and mussel attachment, especially the *E. crassipes* (flower, leaf, stem, and roots) and *T. domingensis* (inflorescence and upper aerial part) extracts. In fact, there is an extreme consensus in the literature that the quantity and quality of chemical compounds can vary among plants due to biotic and abiotic factors, which vary between species (Reigosa et al. 2013; Ramos et al. 2022). However, in our study, we observed that the plant species under investigation is the predominant factor affecting the composition of allelochemicals. A similar response pattern was reported by Morales et al. (2024), who observed that the antibiofilm effect of aquatic macrophyte extracts on marine bacteria varied more between the species studied than between plant organs of the same species.

The concentration and quality of allelochemicals can also vary according to factors such as herbivory, temperature, precipitation, and seasonality, as well as the spatial and biological form of aquatic plants (They et al. 2015; Álvarez-Martínez et al. 2020; Hamidi et al. 2022; Ramos et al. 2022). In the present study, all biotypes of aquatic macrophytes: submerged, floating, and emerging, were assessed. *E. crassipes* is a floating macrophyte with roots below the water surface (Trindade et al. 2010; Thomaz and Esteves 2011), while *T. domingensis* is an emerging species with roots in the sediment and leaves reaching great heights above the water level (Trindade et al. 2010; Thomaz and Esteves 2011). Floating and submerged aquatic macrophytes are generally more sensitive to water quality than emerging ones (Trindade et al. 2018). Sensitivity may be associated with high growth rates and competition for nutrients and light

(Reynolds 2006; Tang et al. 2017). Results from the present study indicate that the extracts from floating and emerging macrophytes had similar effects against individual bacterial species and multispecies community inhibition. The present results are, therefore, in line with Morales et al. (2024), who observed that submerged and emerging aquatic macrophytes had similar effects on estuarine bacteria.

Macrophytes have a wide range of allelochemicals, most frequently phenolic compounds, alkaloids, terpenoids, and fatty acids, which are regularly reported in the literature as having the potential to inhibit algal growth (Li et al. 2021). Although flavonoids are also reported to have antifouling properties (Agostini et al. 2021b), the chemical composition of the macrophyte extracts was not determined. Nonetheless, there is wide evidence in the literature reporting the presence of allelochemicals in these plants (Shanab et al. 2010; Silva et al. 2010; Patel 2012; Lobo et al. 2013). The main allelochemicals present in *E. crassipes* are alkaloids, phenolic compounds, and terpenoids, which have been used as antimicrobial agents against some pathogenic strains of bacteria, fungi, and algae (Shanab et al. 2010; Patel 2012). The macrophyte *T. domingensis* is known for its antimicrobial and anthelmintic properties due to its high concentration of secondary compounds such as flavonoids, tannins, and phenols (Silva et al. 2010; Lobo et al. 2013).

Although several studies have investigated the effects of plant extracts against macrofouling (Feng et al. 2018; Agostini et al. 2021b, 2022; Pérez et al. 2021), those specifically addressing golden mussel are scarce (Agostini et al. 2021b). This study is the first to evaluate the effect of macrophyte extracts on golden mussel byssal attachment. The *E. crassipes* and *T. domingensis* extracts showed attachment inhibition $\geq 80\%$ for this species in terms of size, smaller or larger than 10 mm, which is in line with other studies on the antifouling effect of plant extracts on macrofouling. Pérez et al. (2021) observed the effect of extracts of *Verbena bonariensis* and *Tillandsia tenuifolia* on macrofouling by the adult mussel *Mytilus edulis* (Linnaeus, 1758), while Feng et al. (2018) reported that 15 alkaloids extracted from terrestrial plants were effective in inhibiting the attachment of the larvae of the barnacle *Fistulobalaus albicostatus* (Pilsbry, 1916) and the larvae of bryozoan *Bugula neritina* (Linnaeus, 1758). It must be emphasized that the macrofouling assay carried out with the golden mussel in the present study identified an anti-attachment response; however, this attachment inhibition may have occurred due to the behavioral toxicity of the extracts or other mechanisms of action. Therefore, in future research, mortality tests should be conducted alongside anti-attachment assays.

Natural compounds, in addition to having an antifouling effect for both micro- and macrofouling, must also have low toxicity for non-target organisms (Pérez et al. 2021). In this study, toxicity tests were carried out using organisms representing three different trophic levels, establishing a broad ecological scenario in the aquatic ecosystem. It was observed that for both extracts, there was a difference in the safe dilutions between trophic levels. For the species at the base of the food chain, *P. boryanum*, there was a decrease in cell density and chlorophyll at 50 and 100% dilutions, with a safe dilution of 35%, and the primary consumer (*D. magna*) followed the same pattern. For the species at the highest trophic level, *P. promelas*, the effect of reducing survival and weight was only at 100% dilution, presenting a safe dilution of 70%.

Furthermore, it was observed that microalgae and Cladocera were more sensitive than fish. This was to be expected, as the size of the organism, as well as the study species, can affect sensitivity to chemical substances (Costa et al. 2008). The toxicity of microalgae may be attributed to the mode of action of the chemical substances, mainly involving the inhibition of photosynthesis, also triggering the inhibition of growth (Silva et al. 2024), as found in the present study. Microalgae are important primary producers in aquatic ecosystems, and effects at this level can compromise not only the survival of microalgae but also that of primary and secondary consumers, triggering disturbances at the community level in the ecosystem (Cedervall et al. 2012).

The toxic effects on the cladoceran *D. magna* are also worrying as they are intermediate organisms in the trophic chain, where while they feed on bacteria and algae, they also serve as food for invertebrate and vertebrate predators (e.g., fish) (Thorp and Covich 2009), thus also causing disturbances at the community level. The importance of carrying out toxicity tests at different trophic levels is emphasized as each species may present different sensitivity to chemical substances tested (Costa et al. 2008), besides helping to simulate a natural ecosystem (Pane et al. 2008).

In the present study, it was observed that dilutions of macrophyte extracts $> 35\%$ had toxicological effects on non-target organisms and were dependent on the non-target species. Other studies (Zheng et al. 2010; Techer et al. 2016; Huang et al. 2016) also reported toxic effects at low dilutions of macrophyte extracts on non-target organisms. Specifically, high concentrations of the allelochemicals linoleic acid and salicylic acid extracted from macrophytes were found to be toxic to *Danio rerio* (Hamilton, 1822), *D. magna*, and *Moina macrocopa* (Straus, 1820).

The search for natural antifouling alternatives has increased significantly over the years, especially for those seeking the use of phytochemicals as antifouling agents (Agostini et al. 2021b; Hamidi et al. 2022). Despite this, studies that use extracts from macrophytes as an alternative to control biofouling are still incipient. In fact, although the ecological dynamics of aquatic macrophytes in freshwater ecosystems are very well known, little is known about their antifouling effects. Results from this study suggest that these

plants, especially *E. crassipes* and *T. domingensis*, have great potential to control biofilm formation, inhibiting QS communication between bacteria and attachment of the golden mussel. Improved antifouling activity might be obtained by organic solvent-based extraction with ethanol, ethyl acetate, and/or methanol which would target polar compounds and lipophilic and hydrophilic molecules, respectively. Therefore, chemical compounds present in the macrophytes extracts could be incorporated into paints to develop natural antifouling alternatives that are less harmful to the environment (Pérez et al. 2021; Hamidi et al. 2022).

We reinforce that our study was developed only in the laboratory with tests with individual biofouling organisms, having limitations and not providing insights into the effects of macrophyte extracts on the entire biofouling process at different stages of development. This limitation can be circumvented through future studies with antifouling evaluations in the field, which allow tests to be carried out under environmental conditions of complex interactions between fouling organisms and hydrodynamics of the environment, making the test more similar to natural conditions (Romeu and Mergulhão 2023). In addition, for the incorporation of the compounds present in macrophyte extracts into antifouling solutions (Hamidi et al. 2022), it is necessary to carry out the chemical identification of these extracts in order to search for the main bioactive molecule responsible for this effect. As a result, our efforts are currently already directed to ongoing research on the validation of antifouling effectiveness under in situ conditions and chemical characterization of extracts. Besides, we emphasize that in our study, we did not provide information on the durability of the antifouling effect of the extracts and that studies are needed to understand the half-life of the effects of these extracts.

Conclusions

Of the 25 plant extracts tested here, those from *E. crassipes* and *T. domingensis* were the most promising as they efficiently inhibited > 70% of biofilm formation, inhibited quorum sensing, and inhibited the attachment of golden mussels while being considered safe for non-target organisms at dilutions of 35%. Thus, the potential of aquatic macrophyte extracts to inhibit biofilm through inhibition of QS and inhibition of golden mussel attachment was demonstrated, even at low extract dilutions (≤ 35%). Therefore, extracts from these macrophytes offer a new perspective on developing natural antifouling paints and should eventually be further explored such as the identification of the chemical compounds present in the extracts with the antifouling effect, half-life tests of the extracts to understand the durability of their effect, and field tests to verify the antifouling effect under natural conditions.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s11356-024-35744-y.

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Author contribution All authors contributed to the study conception and design. The authors MLPM and BO contributed to writing, conceptualization, methodology, laboratory analysis, statistical analysis, revision, and editing. PSG, CdMGM, DM, FRB, LB, EB, AS, HC, ROC, NHT, GLLP, and VOA contributed to the conceptualization, methodology, statistical analysis, revision, and editing. The first draft of the manuscript was written by MLPM, and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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Data availability The authors declare that the data supporting the findings of this study are available within the paper and its Supplementary Information files. Should any raw data files be needed in another format, they are available from the corresponding author upon reasonable request.

Declarations

Ethical approval According to Brazilian legislation (Law 11,794 of October 8, 2008), research with invertebrates does not require authorization from any ethics or animal welfare committee. The collection of invertebrates was approved by the Brazilian Agency SisBio (process no. 77095–1). The assay with *P. promelas* was approved by the Consejo Nacional del Ética Animal (CNEA) – Ata n° 17/11.

Consent to participate Not applicable.

Consent for publication Not applicable.

Competing interests The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper. Co-author Ng Haig received research support from the National Council for Scientific and Technological Development – CNPq (Process 404233/2021–0), and co-author Grasiela Lopes Leães Pinho received research support from the Mixed Technical Commission of Salto Grande and Latitud (Uruguay Technological Laboratory Foundation – LATU).

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