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Study of the antagonist interactions between invasive plants from Danube Delta and the associated microbiota

Irina Gheorghe[1], Mariana Carmen Chifiriuc[1]*, Paula Anastasiu[1], Lumita Marutescu[1], Eliza Oprea[2], Veronica Lazar[1]

ABSTRACT

Elodea nuttallii has been introduced from North America to Europe and it is now common in many water bodies where it forms dominating stands. We investigated the antimicrobial potential of *E. nuttallii* against *Enterobacter cloacae* and *Pseudomonas aeruginosa* strains isolated from different submersed macrophytes. Methanolic extracts of this invasive plant inhibited the growth of most of the tested microbial strains. The quantitative assay of the antimicrobial activity was performed by broth microdilution method in order to establish the minimal inhibitory concentration (MIC). The obtained results are leading us to the hypothesis that *E. nuttallii* could be used for the development of novel antimicrobial products.

Keywords: *antimicrobial activity, Elodea nuttallii, plant extract*

1. INTRODUCTION

Since *Elodea nuttallii* (Planch.) St. John was introduced to Europe from North America in 1939, it has invaded many water bodies and is widely spread over the continent (Erhard, D., 2005). Its success is usually related to the ease of adaptation to several environmental factors [1]. The potential of natural compounds of vegetal origin to be used as therapeutical remedies is known for a longtime, but their use is still empirical [1]. The problem of microbial resistance, as well as the negative impact of the chemical substances discharged in the external environment on the ecological balance has reinforced the studies concerning the characterization of the chemical structures of vegetal products and the active doses, aiming to understand their specific mechanisms of action.*E. nuttallii* (Planch.) St. John belongs to *Hydrocharitaceae* family, *Alismatales* order, *Magnoliopsida* class; being a perennial aquatic plant, or submergent macrophyte. The flower have three small white petals, the male flowers with 4.5-5 mm petals and nine stamens, and the female flowers with 2-3 mm petals and three fused carpels. Its fruit is an ovoid capsule, about 6 mm long containing several seeds ripen underwater. The seeds are 4-5 mm long, fusiform, glabrous (round), and narrowly cylindrical. It flowers from May to October [2], it grows rapidly in favorable conditions and can choke shallow ponds, canals and the margin of some slow-flowing rivers. It requires summer water temperatures of 10-25°C and moderate to bright lighting [2]. It is frequently used as an aquarium plant [3]; it contains 7-O-diglucuronides of the flavones luteolin, apigenin and chrysoeriol [4, 5].

The purpose of the present work was to evaluate the bactericidal and antibiofilm efficiency of *E. nuttallii* extracts against *Enterobacter cloacae* and *Pseudomonas aeruginosa* strains isolated from

[1] Department of Microbiology, Faculty of Biology, University of Bucharest, Romania
*Corresponding author e-mail address: ***carmen_balotescu@yahoo.com***
[2] Department of Organic Chemistry, Faculty of Chemistry, University of Bucharest, Romania

the aquatic ecosystems in St. George channel (Danube Delta), for highlighting possible applications of this invasive plant, as source of new active principles with antimicrobial activity.

2. EXPERIMENTAL SECTION

2.1. Plant material. Plant samples of *E. nuttallii* were taken from St. George channel (Danube Delta), Romania in September 2011. Plant material was washed with bi-distilled water to remove dust, soil particles and damaged portions and air-dried in a dark room at constant humidity.

2.2. Preparation of the crude extracts of *E. nuttallii.* Plant material was extracted twice for 2 h in 50% (v/v) aqueous methanol [5]. Aliquots of these extracts were evaporated to dryness and resuspended in 50% methanol to a final concentration of 100 mg plant dry weight per milliliter. Control using only solvent without plant material were prepared in the same way.

2.3. Microbial strains. The antimicrobial activity of the investigated extracts was tested against six strains of *Enterobacter cloacae* and one strain of *Pseudomonas aeruginosa* isolated from the aquatic ecosystems in St. George channel (Danube Delta).

2.3.1. Qualitative screening for the antimicrobial activity. The antimicrobial activity the obtained extract ofwas tested on Mueller-Hinton medium (MH) recommended by the Clinical and Laboratory Standards Institute (CLSI). The bacterial suspensions adjusted to a density corresponding to the 0,5McFarland nephelometric standard, were put in a contact with the extract for 10, 15 and 30 min; as well as with methanol as positive control.

2.3.2. Quantitative assay of minimal inhibitory concentration (MIC). The MIC value was determined by microdilution technique, in 96 multi-well plates. The plates were incubated for 24h al 37°C, and MIC values were determined by the macroscopic evaluation of the wells [5], (as the lowest concentration of the tested extract which inhibited the visible microbial growth) and spectrophotometrically (by measuring the A_{620}nm for the obtained microbial cultures) [6].

2.3.3. Microbial adherence to the plastic substratum represented by 96 multi-well plates. After the performance of the MIC protocol, the plates were emptied, washed three times with sterile saline and fixed with cold methanol for 5 min. The bacterial cells that adhered to the plastic wells were stained with 1% violet crystal solution for 15 min; and the colored biofilm was resuspended in 33% acid acetic solution [7]. The biomass was assessed spectrophotometrically, by measuring the A_{490nm}, for the obtained colored suspension [8-10].

3. RESULTS SECTION

The qualitative screening demonstrated the early microbiostatic activity of the extract against all

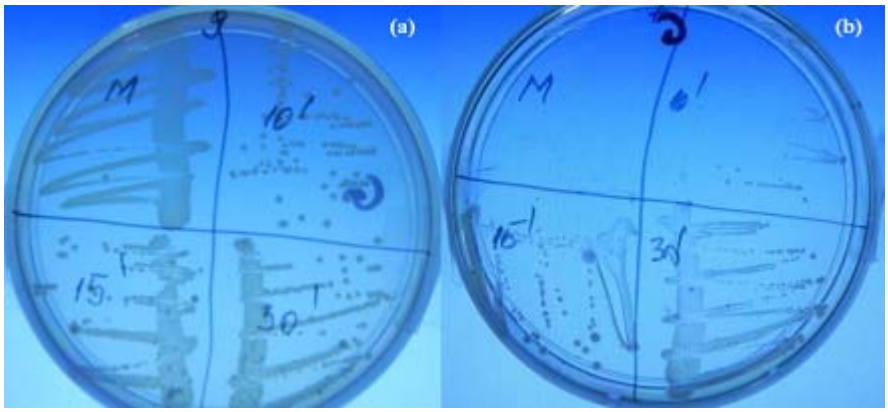

Figure 1: Qualitative screening of the antimicrobial activity, showing the absence of the microbial growth in the presence of methanol and the reduced microbial growth in the presence of *E. nuttallii* plant extract acting for 10 min, 15 min and 30 min, as compared to the untreated culture (M).

tested strains (fig. 1a and 1b), revealed by the inhibition of microbial growth in the presence of the plant extracts as compared to the untreated cultures; the MIC value being situated between 125-31.5µL/mL (fig. 2). The *E. nuttallii* extract inhibited the adherence of *E. cloacae* and *P. aeruginosa* strains, expressed at the MIC/2 concentration of the methanolic extract (fig. 3).

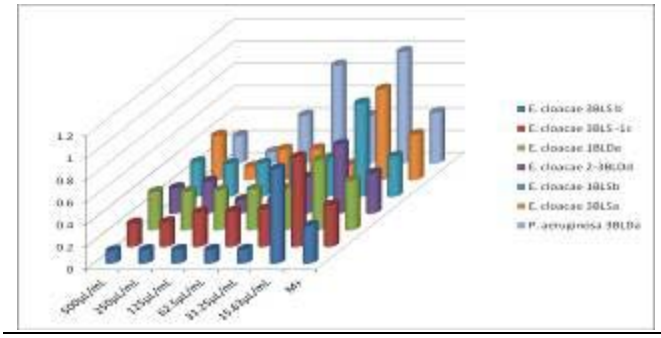

Figure 2: The results of the quantitative assay of minimal inhibitory concentration (MIC) of *E. nuttallii* aqueous extract against the *E. cloacae* and *P. aeruginosa* tested strains by serial microdilution technique.

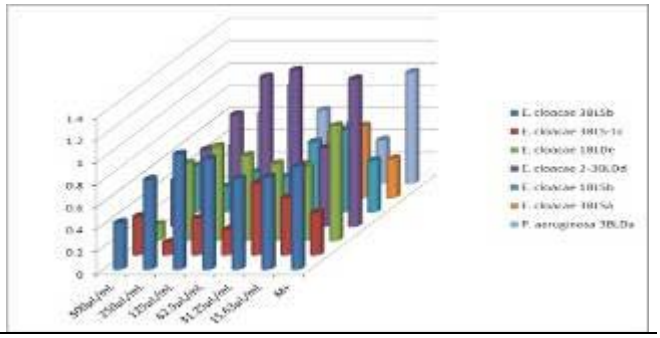

Figure 3: The results of the quantitative assay of of *E. nuttallii* aqueous extract against the *E. cloacae* and *P. aeruginosa* bacterial strains adherence to the plastic substratum represented by 96 multiwell plates.

4. CONCLUSIONS

The study of the natural compounds (chemical structure, mechanisms of action) with therapeutic action from superior medicinal plants is a priority of the international research. Our study showed that *E. nuttallii* contains antimicrobial compounds which are also inhibiting the bacterial adherence to the inert substratum. These results could open new perspectives, related to the understanding of the mechanisms used by this plant to become invasive (e.g. interference with algae and cyanobacteria growth) on one side, and on the other side, to the possibility to exploit this plant for obtaining new antimicrobials.

5. REFERENCES

[1] Bubulica M.V., Anghel I., Grumezescu A.M., Saviuc C., Anghel G.A., Chifiriuc M.C., Gheorghe I., Lazar V., Popescu A., In vitro evaluation of bactericidal and antibiofilm activity of *Lonicera tatarica* and *Viburnum opulus* plant extracts on *Staphylococcus aureus* strains, *Farmacia*, 60, 1, 80-91, **2012**.

[2] Erhard D., Gross M.E. Do environmental factors influence composition of potential allelochemicals in the submersed freshwater macrophyte *Elodea nuttallii* (*Hydrocharitaceae*)? *Verhandlungen der Internationalen Vereinigung fur Limnologie*., 29, 287-291, **2005**.

[3] Erhard D., Gross M.E., Allelopathic activity of *Elodea canadensis* and *Elodea nuttallii* against epiphytes and phytoplankton, *Aquatic Botany*, 85,203-211, **2006**.

[5] Hiscock P., Enciclopedia of Aquarium Plants. Interpret Publishing. United States and Canada. ISB-07641-5521-0, **2003**.

[6] Huxley A., New RHS Dictionary of Gardening. Macmillan ISBN 0-333-47494-5, **2003**.

[7] Limban C., Missir A.V., Chirita I., Neagu A.F., Draghici C., Chifiriuc M.C., Synthesis of new thiourea scaffold compounds, *Rev. Chim*., 62, 168, **2011**.

[8] Muess R., Species specific flavone glucuronides in *Elodea* species. *Biochem. Syst. Ecol*., 11, 261-265, **1983**.

[9] Panus E., Chifiriuc M.C., Banu O., Mitache M., Bleotu C., Rosoiu N., Lazar V. Comparative study o resistance and virulence markers in *Escherichia coli* strains isolated from hospital surfaces, clinical specimens and drinking marine waters, *Biointerface. Res. Appl. Chem*., 1, 24-30, **2011**.

[10] Saviuc C., Grumezescu A.M., Oprea E., Rdulescu V., Dascalu L., Chifiriuc M.C., Bucur M., Banu O., Lazar V., Antifungal activity of some vegetal extracts on *Candida* biofilms developed on inert substratum, *Biointerface Res. Appl. Chem*., 1, 15-23, **2011**.

[11] Grumezescu A.M., Chifiriuc M.C., Marinas I., Saviuc C., Mihaiescu D., Lazar V., *Ocimum basilicum* and *Mentha piperita* essential oils influence the antimicrobial susceptibility of *Staphylococcus aureus* strains, *Lett Appl. NanoBioSci*., 2, 14-17, **2012**.