



POTENTIAL ANTIMICROBIAL ACTIVITIES OF *ZOSTERA* SPP. FROM AEGEAN SEA AGAINST *EDWARDSIELLA TARDA*

H. YAVUZCAN¹, F. S. SEÇER¹, M. ERYILMAZ², S. ÖZBILGIN³,
B. ERGENE³, H. C. TEKIN¹ & G. S. İŞCAN³

¹Department of Fisheries and Aquaculture, Faculty of Agriculture, Ankara University, Ankara, Turkey; ²Department of Pharmaceutical Microbiology, Faculty of Pharmacy, Ankara University, Ankara, Turkey; ³Department of Pharmacognosy, Faculty of Pharmacy, Ankara University, Ankara, Turkey

Summary

Yavuzcan, H. F., S. Seçer, M. Eryılmaz, S. Özbilgin, B. Ergene, H. C. Tekin & G. S. İşcan, 2017. Potential antimicrobial activities of *Zostera* spp. from Aegean Sea against *Edwardsiella tarda*. *Bulg. J. Vet. Med.*, **20**, Suppl. 1, 86–88.

Zostera spp. is one of the most widely distributed plant genera in marine environment which was naturally placed in Urla, İzmir Bay, Turkey. *Edwardsiella tarda* is an infectious agent of Edwardsiellosis in cultured and non-cultured aquatic organisms. In this study we evaluated the antimicrobial activity of extracts of *Zostera* spp. against *Edwardsiella tarda* ATCC 15947. Minimum inhibitory concentrations of ethyl acetate and methanol extracts were found to be 1.25 mg/mL and 5 mg/mL, respectively.

Key words: antimicrobial activity, *Edwardsiella tarda* ATCC 15947, seagrasses, *Zostera* spp.

INTRODUCTION

The study of marine organisms for their bioactive potential has gained momentum in recent years with a growing recognition of their importance to human health. Bioactive chemicals from marine plants, such as phenolic compounds that play important roles for their survival and growth, are now explored for the development of new drugs and health products for humans (Toroğlu & Çenet, 2006; Al-Saif *et al.*, 2014). *Zostera* spp. is one of the most widely distributed plant genera with contributing to nutrient cycling and sediment stabilisation, and providing food stuffs

and habitat for many marine organisms. *Edwardsiella tarda* is one of the most frequently isolated pathogens in aquaculture organisms. Many investigations revealed that *Edwardsiella tarda* is an infectious agent of Edwardsiellosis in cultured and non-cultured aquatic organisms (Buller, 2004). Infectious diseases account for high percentages of aquaculture production lost as result of disease.

The aim of this study was to detect potential antimicrobial activity of the dead leaves of *Zostera* spp. against *E. tarda*.

MATERIAL AND METHODS

Seagrass material get from dead leaves of *Zostera marina* and *Z. noltii* were collected from Urla district near Izmir Bay (Lat 38° 31' N and Long 26° 69' E) in autumn. Taxonomic identity of the plants was confirmed by reference to literature (Chapman & Chapman, 1973; Stewart & Fairfull, 2007; Guiry & Guiry, 2014).

Extracts were prepared by maceration at room temperature successively using n-hexane, ethyl acetate and methanol. All solvents were evaporated under vacuum and the dried residues were dissolved in DMSO/water (20% v/v) before testing for their antimicrobial activities (Bahadır *et al.* 2010; Saltan Çitoğlu *et al.*, 2010).

The fractions were screened *in vitro* for their potential antibacterial activities against *Edwardsiella tarda* ATCC 15947. Micro broth dilution method was used for determination of the minimum inhibitory concentrations (MIC). The cultures were obtained in Mueller Hinton Broth (Difco, Difco Laboratories, Detroit, MI, USA) supplemented with 2% NaCl. Serial two-fold dilutions from 10 to 0.078 mg/mL were prepared in medium. A set of wells containing only inoculated broth were used as control. After incubation for 18–24 h at 35±1 °C, the last well with no microbial growth was recorded to represent MIC value (mg/mL) (Wayne, 2008; 2009).

RESULTS AND DISCUSSION

MIC values (mg/mL) of *Zostera* spp. extracts against *Edwardsiella tarda* ATCC

15947 are presented in Table 1.

Aquaculture is characterised by being a fast-growing food sector with providing economic value by production and employment. However, intensification of aquaculture has led to the promotion of conditions that favour the development of diseases which is one of the main obstacles in constant development of aquaculture and its sustainability. In controlling the infectious diseases disinfectants and antimicrobial use are traditional methods but, the revolutionised therapy of infectious diseases by the use of these agents have certain limitations. It is known that bacterial resistance developed due to selective pressure on microorganisms by antimicrobial drugs use over time. Nowadays, several alternatives such as vaccination and immunostimulants instead of antimicrobial drug could not be efficacious. The solution strategies of environmental-friendly prophylactic and preventive methods based on immune system of the organism had no impact as expected due to the high diversity in pathogen and fish species under culture. Environmental-friendly alternatives are still needed to maintain a healthy microbial environment in aquaculture systems. Thus, an alternative solution for the elimination of potential pathogens by developing a strategy to decrease of bacterial load in any marine environment/organisms using dead leaves of *Zostera* spp. can be considered in aquaculture.

Experiments showed that many microalgae and *Staphylococcus aureus* development was inhibited by *Zostera marina*

Table 1. MIC values (mg/mL) of *Zostera* spp. against *Edwardsiella tarda* ATCC 15947

Test microorganism	Extracts (mg/mL)		
	N-hexane	Ethyl acetate	Methanol
<i>Edwardsiella tarda</i> ATCC 15947	—*	1.25	5.0

extract (Harrison & Chan, 1980). *Zostera marina* and *Zostera noltii* phenolic acid compounds in their extracts, protects their marine origin fungal diseases (Achamlale *et al.*, 2009). Recent studies on *Gracilaria*, *Acanthospora*, *Ulva*, *Sargassum* and *Padina* extract was effective on pathogen bacterial agents (Thirunavukkarasu *et al.*, 2013). There has not been observed in a study using *Zostera marina* and/or *Zostera noltii* extracts as bioactive agents for the inhibition of bacterial pathogens of fish disease. This is the first record of the *Zostera* spp. extracts MIC values uses for *Edwardsiella tarda*.

In conclusion, the ethyl acetate and methanol extracts of *Zostera* spp. are of antimicrobial activity to *E. tarda* thus, it seems possible to use the freshly dead leaves of seagrass to antimicrobial agent for fish pathogens.

ACKNOWLEDGEMENT

Authors greatly thank TÜBİTAK (*The Scientific and Technical Research Council of Turkey*) for the support of Project 114Y141.

REFERENCES

- Achamlale, S., B. Rezzonico & M. Grignon-Dubois, 2009. Evaluation of *Zostera detritus* as a potential new source of zosteric acid, *Journal of Applied Physics*, **21**, 347–352.
- Al-Saif, S. S. A., N. Abdel-Raouf, H. A. El-Wazanani & I. A. Aref, 2014. Antibacterial substances from marine algae isolated from Jeddah coast of Red sea, Saudi Arabia, *Saudi Journal of Biological Sciences*, **21**, 57–64.
- Bahadır, Ö., G. Saltan Çitoğlu, K. Šmejkal, S. Dall'Acqua, H. Özbek, J. Cvacka & M. Zemlicka, 2010. Analgesic compounds from *Scorzonera latifolia* (Fisch. and Mey.) DC., *Journal of Ethnopharmacology*, **131**, 83–87.
- Buller, N. B. 2004. Bacteria from Fish and Other Aquatic Animals: A Practical Identification Manual. CABI. pp. 361.
- Chapman, U. J. & D. J. Chapman, 1973. The Algae, 2nd edn, The Macmillan Press Ltd. Hong Kong.
- Guiry, M. D. & G. M. Guiry, 2014. AlgaeBase. World-wide electronic publication, National University of Ireland, Galway. <http://www.algaebase.org/> (Date last accessed 18 March 2014).
- Harrison, P. G. & A. T. Chan, 1980. Inhibition of the growth of micro-algae and bacteria by extracts of eelgrass (*Zostera marina*) leaves, *Marine Biology*, **61**, 21–26.
- Saltan Çitoğlu, G., Ö. Bahadır & S. Dall'Acqua, 2010. dihydroisocoumarin derivatives isolated from the roots of *Scorzonera latifolia*, *Turkish Journal of Pharmaceutical Sciences*, **7**, 205–212.
- Stewart, M. & S. Fairfull, 2007. Seagrasses, *Primefact*, **629**, 1–8.
- Thirunavukkarasu, R., P. Pandiyan, D. Balaraman, K. Subramaniyan, G. E. G. Jothi, S. Manikkam & B. Sadaiyappan, 2013. Isolation of bioactive compound from marine seaweeds against fish pathogenic bacteria *Vibrio alginolyticus* (VA09) and characterisation by FTIR, *Journal of Coastal Life Medicine*, **1**, 26–33.
- Toroğlu, S. & M. Çenet, 2006. Tedavi amaçlı kullanılan bazı bitkilerin kullanım alanları ve antimikrobiyal aktivitelerinin belirlenmesi için kullanılan metodlar, *KSÜ, Fen ve Mühendislik Dergisi*, **9**, 2.
- Wayne, P. A., 2008. Reference method for broth dilution antifungal susceptibility testing of yeasts, *Clinical and Laboratory Standards Institute*, Approved standard-third edition, M27–A3.
- Wayne, P. A., 2009. Method for antifungal disk diffusion susceptibility testing of yeasts, *Clinical and Laboratory Standards Institute*, Approved guideline-second edition, M44–A2.