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Cytotoxic Activity of Solvent Extracts of Detrital *Zostera* spp. Leaves Collected from West Coast of Turkey

Hijran Yavuzcan Yildiz[1*], Ozlem Bahadir Acikara[2], Mujde Eryilmaz[3], Serkan Ozbilgin[2], Burcin Ergene[2], Gulcin Saltan Iscan[2]

[1]*Department of Fisheries and Aquaculture, Ankara University, Faculty of Agriculture, Ankara, Turkey*

[2]*Department of Pharmacognosy, Ankara University, Faculty of Pharmacy, Ankara, Turkey*

[3]*Department of Pharmaceutical Microbiology, Ankara University, Faculty of Pharmacy, Ankara, Turkey*

Corresponding Author: *Hijran Yavuzcan Yildiz, *Department of Fisheries and Aquaculture, Ankara University, Faculty of Agriculture, Ankara, Turkey*

Abstract: *Seaweeds represent valuable sources of bioactive compounds with different potential applications in pharmaceutical industries. Solvent extracts of detrital leaves of seaweed, Zostera spp. found as the beach-cast in Aegean Coasts of Turkey was assayed for theircytotoxic activity. Extracts of methanol, ethyl acetate, and n-hexane of detrital leaves were used to determine the cytotoxicityactivities by the brine shrimp cytotoxicity test. LC50 48 h values of the extracts of Zostera spp. toArtemia nauplii varied by the solvent used. Cytotoxic activity of the solvent extracts used in this study were in the following order: ethyl acetate> hexane> methanol. The results of this investigation elicited that the detrital leaves of Zostera spp. have the cytotoxic potential, evidencing the existence of bioactive compounds in the beach-cast seaweed.*

Keywords: *Sea Weed, Zostera spp, solvent extract, cytotoxicity, brine shrimp*

1. INTRODUCTION

Bioactive compounds produced by marine plants are under extensive investigation as a new renewable source for pharmaceutical and biotechnological applications [1]. Seeweeds are the eukaryotic organisms that live in marine environment and recognized as a potential source of bioactive substances [2]. Marine plants are known to produce a plentiful and varied form of secondary metabolites characterized by a extensive range of biological activities [3-5].Seaweed of the genus *Zostera* spp.is widespread around the World with providing large amounts of biomass. *Zostera* spp. is of ecological importance in maintaining healthy estuarine and coastal ecosystems with taking a part in nutrients cycle [6]. In coastal marine plants,the decomposition of detrital components is not quick, providing the conservation of organic matter and available nutrients for a long time [7]. The strength of marine plants against decomposition in relation to secondary metabolites in detrital leaves is of interest to evaluate the potential of the seaweeds as renewable source for the production of bioactive substances [8]. The compounds with cytotoxic, antiviral, antihelmintic, antifungal and antibacterial activities have been detected in green, brown and red algae [9-11]. Marine natural products yielding bioactive compounds such as marine invertebrateanimals, algae, fungi and bacteriawere reviewed for their antitumor capacity and cytotoxic properties by Mayer and Gustafson [12]. The research on cytotoxic effects of the bioactive compounds of seaweeds is relatively scarce. Ara *et al.*[9] screened the cytotoxic activity of 13 brown, 6 green and 3 red seeweed species. Vinayak *et al.* [13] screened seven brown seaweeds by their cytotoxic activities using the brine shrimp assay with indicating the existence of potent cytotoxic compounds. Iyapparaj *et al.*[14] studied *Syringodium isoetifolium* and *Cymodocea serrulata* for their toxic properties.

The present study was undertaken to evaluate the cytotoxic activities of methanol, ethyl acetate, and *n*-hexane extracts of detrital *Zostera* spp. leaves found as beach-cast. The cytotoxic effect of the *Zostera* spp. extracts was firstly investigated by *Artemia* nauplii lethality test in the present study.

2. MATERIALS AND METHODS

2.1. *Zostera* spp.Detritus Samples

Zostera spp. leaves deposited on the beach were collected from the coasts of Izmir, Aegean Sea, Turkeyat the coordinates of Lat. 09˚ 17.417'N; Long. 079˚ 08.558'E at the beginning of June. Dead leaves of seaweed were immediately transferred to the laboratory and washed thoroughly with tap water to detract from outer materials and air-dried.The samples in powder form were kept in laboratory refrigerator until use.

2.2. Extraction and Isolation

Zostera spp. (2,270 g) samples were throughly powderised beforeextracting by maceration (20 L). The extract was filtered and densification under reduced pressure to pick up the crude methanol extract (638.82 g). The methanolic extract was suspended in distilled water: methanol (1:1) mixture and fractionated by liquid-liquid extraction succesively with *n*-hexane, and ethyl acetate, respectively. Methanol, ethly acetate and n-hexane were used as solvents due the fact that*i*)*n*-hexane allows to extract non-polar compounds (terpenoids, some aglycones, etc.), medium polar compounds such as most of the aglycones (flavonoids, coumarins etc.); *ii*) ethyl acetate to extract glucosides;*iii*) methanol to extract polar compounds (polyphenols, glycosides). Each fraction was concentrated to dryness under reduced pressure by evaporation to yield n-hexane fraction (23.30 g), ethylacetate fraction (8.88 g) and remaining methanol fraction (521 g). Each fraction was tested for their cytotoxic activities.

2.3. Cytotoxicity Assay

Gnotobiotic Artemia culture and decapsulation procedure were done by following the method of Sorgeloos *et al.*[15]. The cysts of brine shrimp (*A. salina*) were hatched in the Falcon tubes filled with artificial sterilized seawater in 48 hours. Ten active larvae (I instar) were collected by using capillary glass tube and placed in a Petri dishes containing extracts and artificial seawater.

The toxicity of the ethanol extract was determined with a brine shrimp toxicity bioassay as described byCaldwell *et al.*[16]. The extract was resolved in DMSO andprepared in the concentrations of 50, 25, 12.5, 6.25, 3.12, 1.56, 0.78 and0.39 µg in a ml artificial seawater. Ten instar I Artemia were put in each Petri plate and kept under ambient light for 48 h, then the number of dead nauplii was counted under a stereomicroscope. Control groupswere treated in a similar way withoutthe extract to the seawater and with DMSO only. Triplicate tests were performed. The lethal concentration of the extract was assessed as the concentration resulted in 50% mortality of the nauplii (LC50).

3. RESULTS AND DISCUSSION

The percentage mortality caused by various concentrations of seagrasses was taken as log dose against nauplii of *A. salina*. From the present data, it is revealed that dead leaves of *Zostera* spp. showed the maximum cytotoxicity with LC50 48 h value of 156.057 µg/ml for ethyl acetate extract (Fig 1), followed by *n*-hexane extract with LC50 48 h 478.548 µg/ml (Fig 2) and minimum cytotoxicity with LC50 48 h values of 1063.596 µg/ml for methanol extracts (Fig 3).

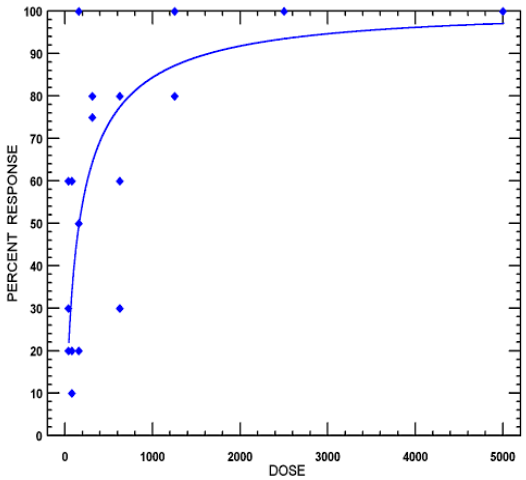

Fig1. *Cytotoxicity activity of Zostera spp. extracts in ethyl acetate using brine shrimp lethality assay.*

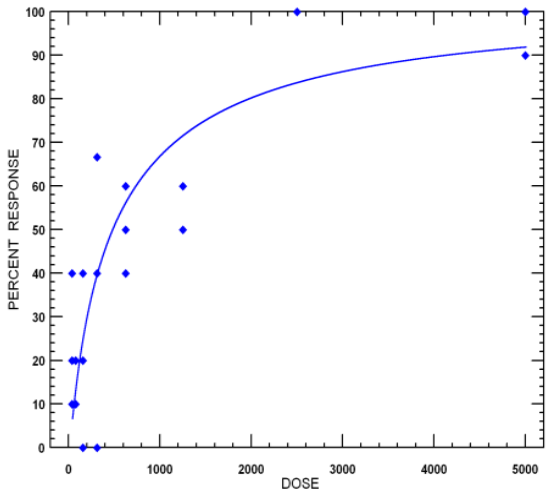

Fig2. *Cytotoxicity activity of Zostera spp. extracts in n-hexane using brine shrimp lethality assay.*

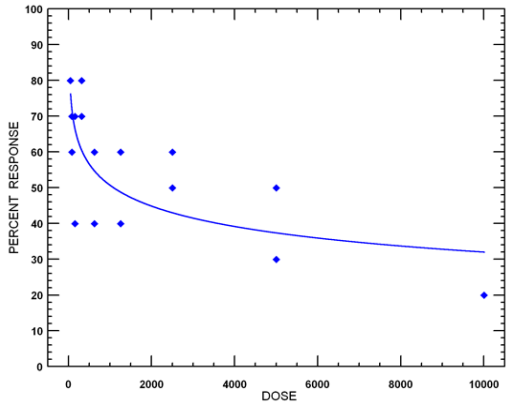

Fig3. *Cytotoxicity activity of Zostera spp. extracts in methanol using brine shrimp lethality assay*

Brine Shrimp Lethality Assay (Artemia) is the applicable system for monitoring biological effects of various plant species. Cytotoxicity and cytostaticity of marine plant, *Ulva lactuca* were reported by Holdt and Kraan [17]. In current study, cytotoxicity of detrital seaweed, *Zostera* spp. was detected in methanol, ethyl acetate and *n*-hexane extracts. Here, the results of cytotoxic activity assessed by *Artemia* nauplii lethality assay have shown that LC50 values of the extract of *Zostera* spp. for 48 h differed by the solvent used in the extraction phase. Ethyl acetate extracts were the most active with LC50 value of 156.057 µg/ml. Cytotoxic activity of the extract solvents used in this study was in the following order; ethyl acetate> hexan> methanol. Seaweedsexhibit different cytotoxicity by the seaweeds species. Kannan *et al.*[18] reported that cytotoxicity of seagrass, *Syringodium isoetifolium* extracts against nauplii of *Artemia salina* was LC50 value of 699.096 µg/ml and for *Cymodocea rotundata* LC50 value was 132.469 µg/ml. Vinayak *et al.*[13] studied methanolic extracts of seven brown seaweeds for their cytotoxic activity using the brine shrimp *A. salina*. The seaweeds; *Dictyopteryisaustralis, Sargassum marginatum, S. variable, S. aspermum* were screened as highly cytotoxic at 100 µg/ml at 18 and 24 h thus,dose-dependant activity was reported as the significant factor in cytotoxicity assays. Manilal *et al.*[19] studied the cytotoxic potentials of red alga, *Laurencia brandenii* with reportingitspossible use in drug development due to existence of cytotoxic secondary metabolites. Studies on cytotoxicity of *Zostera* species do not exist in the previous literature.From the results of this study, LC50 values of *Zostera* spp. extracts for 48 h can be screened mildly cytotoxic for ethyl acetate and *n*-hexan extracts thus, methonolic extracts exhibited cytotoxic activity at high doses. Thus, Iyapparaj *et al.*[14] reported that the LC50 values of methanolic extracts of *S. isoetifolium* and *C. serrulata* were 732 µg/ml and 394 µg/ml, respectively. These values were lower than the values found in the present study for methanolic extracts of *Zostera* spp. (1063.596 µg/ml). The different values of cytotoxicity for different solvent extracts may be related with the bioactive compounds differing in polarity, as stated by Ara et al. [9]. Thus, the stronger cytotoxic activity of ethyl acetate extract may be in relation to the associated bioactive substances with different polarity in the ethy acetate extract from detrital *Zostera* spp. leaves.

4. CONCLUSION

Ethyl acetate extracts of detrital of *Zostera* spp. leaves may possibly contain more bioactive components that are likely to induce cytotoxic activity as detected in *in vitro* brine shrimp toxicity bioassays. Methonolic extracts exhibit low cytotoxic activity, suggesting the less bioactive constituents in methanolic extracts. *n*-hexane extracts exhibited cytotoxicity at mild concentrations. The cytotoxicity activity in methanol extracts was detected at higher concentrations. From the results obtained in the present study, it can be concluded that the biactive substances found in detrital leaves have the cytotoxic activity in a dose-dependant manner.The present study reveals that dead leaves of *Zostera* spp. could be used as a source of the bioactive constituents for their cytotoxic potential. We presented the availability of the potential bioactive constituents with cytotoxic properties in dead biomass of seaweed, *Zostera* spp. This paves the way to new experimental research towards identification and characterization of individual bioactive compounds exhibiting cytotoxic activity.

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