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Research Article

Biological Activity of Red Pitahaya Extracts on *Lactococcus garvieae* and *Vibrio alginolyticus*

Irem CELIK [1], Meltem ASAN-OZUSAGLAM *[1]

[1] Department of Molecular Biology and Genetics, Faculty of Science and Letters, Aksaray University, Aksaray, Türkiye

*Corresponding author e-mail: meltemozusaglam@gmail.com

ABSTRACT

The enhancement population of the world every day brings with it the demand for protein needs. In our study, it was aimed to identify the utilization potential of red pitahaya fruit obtained from Turkey as a natural feed additive. The disc diffusion assay, micro-dilution method for determination of minimum inhibitory (MIC) and minimum bactericidal (MBC) concentration values of the extracts were used to identify the biological activity of the extracts from red pitahaya. The red pitahaya pulp methanol extract against *L. garvieae* and *V. alginolyticus* showed 10.61 mm and 7.65 mm of inhibition zone diameters. The methanol extract of red pitahaya peel has 10.18 mm and 11.25 mm inhibition zone diameters on *L. garvieae* and *V. alginolyticus*. MIC values were determined as 20 µg/µl and 40 µg/µl for pulp methanol extract and as 80 µg/µl and 40 µg/µl for peel methanol extract against *L. garvieae* and *V. alginolyticus*. MBC values of pulp and peel methanol extracts against fish pathogens were determined as 80 µg/µl. As a result, the red pitahaya extracts may have usage potential as natural antimicrobial agents or feed additive in aquaculture.

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INTRODUCTION

In parallel with the rapid increment in the human the world's population, the need for animal protein is also increasing rapidly (Bilgüven and Can 2018). Although this situation makes it necessary to seek for various alternative foods, protein sources such as fish are among the important sources that come to mind in Turkey, which is surrounded by water on three sides (Kocatepe and Turan 2018). Therefore, aquaculture plays a significant role in meeting the increasing protein food request (Godfray et al. 2010). The spread of diseases in aquaculture can be caused by poor environmental conditions, including feeding conditions, poor management, stocking, malnutrition (Quesada et al. 2013). Such conditions can lead to bacterial infections. Antimicrobial agents should be used in aquaculture for the therapy of contagious diseases. Antibiotics are used as therapeutic substance in aquaculture (Romero et al. 2012; Quesada et al. 2013). Antibiotics used in treatment can be found in trace amounts in the animal product consumed. Such antibiotic residues or their metabolites pose an important threat to human health (Bondad-Reantaso et al. 2023).

Vibriosis is one of the most common and devastating diseases in seafood aquaculture, causing significant mortality and economic losses in both fish and shellfish cultures worldwide (Frans et al. 2011). *V. alginolyticus* is one of the pathogens that cause vibriosis and fish deaths in Mediterranean aquaculture (Sanches-Fernandes et al. 2022; Balebona et al. 1998; Chen et al. 2000; Cai et al. 2006). *L. garvieae* is common in rainbow trout (*Oncorhynchus mykiss*) causing lactococcosis and can cause great economic losses (Meyburgh et al. 2017).

Because of their better biodegradability than synthetic materials, plants can also reduce treatment costs or minimize the possibility of bacteria to develop drug resistance, in addition to their alternative uses to antibiotics (Reverter et al. 2014; Salikan et al. 2020; Hassan et al. 2020). Pitahaya is a type of climbing cactus belonging to the genus *Hylocereus* sp. of the *Cactaceae* family. Pitahaya's fruit is juicy and delicious and has gained worldwide recognition (Gunasena et al. 2007; Pannison et al. 2021; Nizamlıoğlu et al. 2021. Pitahaya is widely grown in tropical regions around the world. Red pitahaya fruit, which has started to be cultivated in Turkey (especially in Antalya) in recent years, attracts the attention of consumers with its different fruit structure and color. It has pharmacological values due to the bioactive components it contains. It has been reported to help treat obesity, cancer, type 2 diabetes and other metabolic syndromes (Ramli et al. 2014; Nishikito et al. 2023).

The purpose of the study is to obtain the potential of using red pitahaya from Turkey as a natural additive instead of products containing synthetic additives. In the present study, the potential for use of methanol extracts of red pitahaya as natural feed additives in aquaculture was determined.

MATERIAL AND METHODS

Red Pitahaya Extraction

The red pitahaya (*Hylocereus polyrhizus*) was obtained from the production greenhouse in Antalya-Turkey. Then, the pulp is separated from the peel, and they left to dried. After the grinding process, the powder obtained from red pitahaya pulp and peel (10 g) were extracted with of 30 ml methanol (99.7%) using a sonication device on ice for a total of 40 minutes (10 minutes in 2 repetitions) in 2 days. The red pitahaya extracts were obtained by using a rotary evaporator. The red pitahaya methanol extracts were dissolved in DMSO (Dimethyl Sulfoxide) and sterilized with sterile filters (0.45 µm). They were stored at 4°C throughout the study. For the study, an average of 300-400 g of fruit was used. The tests were performed in triplicate.

Disc Diffusion Susceptibility Assay

The inhibitory effect of pulp and peel methanol extracts obtained from red pitahaya against *Lactococcus garvieae* and *Vibrio alginolyticus* were determined using disc diffusion susceptibility assay (Murray et al. 2006). *L. garvieae* and *V. alginolyticus* test microorganisms were cultured at 25ºC for 24 hours in TSB and TSB/NaCl medium, respectively. The test bacteria were washed with a saline solution (twice). The prepared bacterial suspension (0.5-McFarland) was inoculated on specific agar medium using spread plate method and sterile discs (Diameters: 6 mm) were placed on the agar. The red pitahaya methanol extracts prepared at a

concentration of 200 mg/ml were dripped onto the sterile discs. Kanamycin (K; 30 µg/disc) antibiotic discs was used as controls for pathogenic microorganism strains. The culture dishes were incubation period for 24 hours at the suitable temperatures indicated previously. Then, the zone of inhibition was measured using caliper.

Microdilution Method

The minimum inhibitory (MIC) and bactericidal (MBC) concentrations of red pitahaya extracts were obtained by microdilution method (Chandrasekaran and Venkatesalu 2004). The methanol extracts were added to liquid medium and diluted by a two-fold serial dilution method to obtain a final concentration of 160-10 µg/µl. The microbial suspension was added to each tube and then incubated under the conditions required for each microorganism as mentioned above. After incubation, the extract concentration in the tube without microbial growth was determined according to turbidity and the lowest concentration was recorded as the MIC value. MBC or MFC values were determined by inoculating samples from the mixture onto agar medium. The culture dishes were incubation period at the appropriate temperature for 24 hours. The lowest concentration without eventually growth of incubation was defined as MBC values.

RESULTS AND DISCUSSION

Despite many challenges, production in the aquaculture continues to increase rapidly. Among the difficulties encountered in production, microbial infections cause serious economic losses (Assefa and Abunna 2018). Antibiotics used in aquaculture increase the likelihood of residual antibiotics in fish and aquaculture (Cabello 2006). In this context, using plants in aquaculture may provide an alternative solution. Scientific evidence is increasing on the beneficial effects of plant extracts, which are very rich in natural components, on fish health and the prevention of epidemic diseases (Reverter et al. 2014). In the present study, antimicrobial activity of red pitahaya pulp and peel extracts at 4 mg/disc (20 µl) concentration against *L. garvieae* and *V. alginolyticus* was investigated by disc diffusion susceptibility assay (Table 1). The inhibition zone diameters against *L. garvieae* and *V. alginolyticus* in the pulp extract were determined as 10.61 mm and 7.65 mm. In the peel extract, the inhibition zone diameters were determined as 10.18 mm and 11.25 mm.

Table 1. *Disc diffusion susceptibility assay results of red pitahaya extracts*

Fish Pathogens	**Inhibition zone diameter (mm±SD)**		
	Red Pitahaya Extracts		**Antibiotic**
	Pulp	**Peel**	**K**
Lactococcus garvieae	10.61±0.51	10.18±1.19	17.39±0.95
Vibrio alginolyticus	7.65±0.09	11.25±0.66	18.27±1.47

*K: Kanamycin

The literature studies of red pitahaya extracts against fish pathogens are limited. In a study conducted in 2014, the antimicrobial activity of *Hylocereus polyrhizus* core methanol extract obtained in Bangladesh against *Vibrio* sp. was determined by the well diffusion method. The diameter of the inhibition zone was obtained as 10.30 mm (Tahera et al. 2014). The results of our current study indicated that the peel methanol extract of red pitahaya showed higher antimicrobial activity against *V. alginolyticus* compared to the core methanol extract. In our previous study investigating the biological activity of methanol extracts obtained from white pitahaya pulp and its peel, the results of the disc diffusion test against *L. garvieae* were determined as 9.18 mm and 10.34 mm (Celik and Asan-Ozusaglam, 2022). When compared our current study with our previous study, the antibacterial activity of the red pitahaya fruit methanol pulp extract on the *L. garvieae* pathogen was found to be higher than the white pitahaya methanol fruit pulp extract. The red pitahaya peel extract showed antibacterial activity similar to white pitahaya peel extract against this pathogen. The red pitahaya fruit contains substances with antibacterial content such as alkaloids (betalain pigments), flavonoids and vitamin C, and its peel contains flavonoids, alkaloids and terpenoids. Phenolic compounds can prevent bacterial growth by damaging the cytoplasmic membrane and proteins and can also inactivate some bacterial enzymes (Amalia et al. 2014). In the current study, the antimicrobial activity of red pitahaya extracts may be due to these reasons.

The biological activity of a compound is generally defined as the MIC, the lowest concentration of the compound capable of inhibiting the growth of the microorganism (Mann and Markham 1998). The MBC is the lowest concentration that reduces the number of microorganisms in the medium containing the microbial solution by 99.9 (Kowalska and Dudek 2021). The micro-dilution test results of red pitahaya methanol extracts against *L. garvieae* and *V. alginolyticus* are presented in Table 2. MIC values against *L. garvieae* were determined as 20 µg/µl and 80 µg/µl in pulp and peel extracts. Against *V. alginolyticus*, it was determined as 40 µg/µl in pulp and peel extract. The MBC values of the red pitahaya pulp and peel extracts against *L. garvieae* and *V. alginolyticus* were determined as 80 µg/µl.

Table 2. *Microdilution assay of red pitahaya extracts*

Fish Pathogens	MIC (µg/µl)		MBC (µg/µl)	
	Pulp	**Peel**	**Pulp**	**Peel**
Lactococcus garvieae	20	80	80	80
Vibrio alginolyticus	40	40	80	80

Hendra et al. (2019), determined the biological activity of n-hexane and ethyl acetate extracts obtained from red pitahaya fruit peel against *V. alginolyticus* by micro-dilution method. The growth inhibition (%) of the red dragon fruit n-hexane and ethyl acetate extracts were determined as 72.6 and 74.2. It has been suggested that the differences in antimicrobial activities may be due to the extraction method or may be due to the solvents used (Fidrianny et al. 2017).

CONCLUSION

As the day pass, the increment in the population leads to an increase in the demand for food. Aquaculture has become a very important industry to meet the protein food demand. Intensive production conditions cause microbial infections and serious economic losses are experienced. Antibiotic-added feeds used in aquaculture pass into the human food chain and cause the formation of antibiotic resistance genes. Therefore, the use of plant extracts in aquaculture can be an alternative solution. It is known that pitahaya, which is one of the tropical fruits that has gained popularity especially in our country, is a fruit rich in nutritional value. In our current study, it was determined that methanol extracts of red pitahaya pulp and peel exhibited good antimicrobial activity against the tested microorganisms. The study results have the potential to advanced new literature studies. As a result, it has been reported that red pitahaya extracts may have the potential to be used as natural feed additives in aquaculture.

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Conflict of interest

The authors do not declare any conflicts of interest in the study.

Author contribution

All authors have contributed equally.

Ethical approval

During the writing process of the study titled "***Biological Activity of Red Pitahaya Extracts on Lactococcus garvieae and Vibrio alginolyticus***", scientific rules, ethical and citation rules were followed; No falsification has been made on the collected data and this study has not been sent to any other academic media for evaluation. Ethics committee approval is not required.

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