Journal of Agricultural Production (2020) 1(1): 5-7 https://agriprojournal.com/
e-ISSN: 2757-6620 agriprojournal@gmail.com

JOURNAL OF AGRICULTURAL PRODUCTION

RESEARCH ARTICLE

Investigation of Antibacterial Activity of Two Different Medicinal Plants Extracts Against Fish Pathogens

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ARTICLE INFO

Article History:
Received: 19.12.2020
Accepted: 24.12.2020
Available Online: 31.12.2020

Keywords:
Medicinal plants
Aqueous methanolic extract
Antibacterial activity
Minimum inhibitory concentration

ABSTRACT

Diseases are one of the leading factors affecting the sustainability of aquaculture industry. Due to the undesirable effects of the methods used in the prevention or treatment of diseases, the usability of herbal products has been investigated recently. Thus, in the present study, we aimed to investigate the effects of aqueous methanolic extracts of two different medicinal plants (*Laurus nobilis* and *Brassica nigra*) against *Vibrio anguillarium*, *Yersinia ruckeri*, *Pseudomonas putida*, and *Aeromonas hydrophila* by using minimum inhibitory concentration (MIC). The MIC values of leaf aqueous methanolic extract of *Laurus nobilis* for Aeromonas *hydrophila* and leaf aqueous methanolic extract of *Brassica nigra* for *Vibrio anguillarium* were determined as 3.125 µg ml^{-1} and 100 µg ml^{-1}, respectively. The results showed that *Laurus nobilis* could be used against *Aeromonas hydrophila* and *Brassica nigra* against *Vibrio anguillarium*. Further *in vivo* studies should be conducted to evaluate the usability of these plants.

Please cite this paper as follows:

Karga, M., Kenanoğlu, O. N. and Bilen, S. (2020). Investigation of Antibacterial Activity of Two Different Medicinal Plants Extracts Against Fish Pathogens. *Journal of Agricultural Production*, 1(1): 5-7.

Introduction

Aquaculture industry is one of the most fast growing food industry in the world (Fisheries, 2018). Increased technology could give opportunities to the fish farmers to increase their production rate (Bilen et al., 2013). However, diseases seem to be most limiting factor in the industry. Increasing stoking density and the different technology usage may trigger stress conditions and this can favour occurrence and the spread of the bacterial diseases (Fazio et al., 2013).

Among the bacterial pathogens *Vibrio anguillarium*, *Yersinia ruckeri*, *Pseudomonas putida* and *Aeromonas hydrophila* are opportunistic and ubiquitous, and most commonly infect not only freshwater fish species but also marine fishes. Antibiotic have been mostly used to prevent or treat fish from those pathogens (Corum et al., 2020; Terzi et al., 2020). In some cases, vaccine usage also gives an opportunity to overcome the problem. However, antibiotics have some adverse effect on animal and the environment (Capkin et al., 2015), and vaccines are used for specific pathogens.

Medicinal plants extracts already discovered to cure the fish against diseases (Bilen and Elbeshti, 2019; Bilen et al., 2019) or protect them from many different fish diseases (Bilen et al., 2020a; Bilen et al., 2020b; Bilen et al., 2014) and even in some cases as reproductive promoter (Sonmez et al., 2019). Also, medicinal plants have growth performance and immune system activation in fishes (Amhamed et al., 2018; Bilen et al., 2020c; Elbesthi et al., 2020; Mohamed et al., 2018). Laurel (*Laurus nobilis)* is a tree and has been used for its astringent, healing and diuretic properties (Nayak et al., 2006). Laurel has also antimicrobial and antibacterial effects (Digrak et al., 2001). Black mustard (*Brassica nigra*), native to the southern Mediterranean region of Europe, which has been cultivated for thousands of years. In a previous study, addition of a medicinal plant, *Brassica nigra*, to *Oreochromis niloticus* food improves both the immune and biotransformation systems after

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exposure to a polycyclic aromatic hydrocarbon, BaP (Abbas et al., 2016).

In the present study, we performed to demonstrate that the effects of aqueous methanolic extract of *Laurus nobilis* and *Brassica nigra* against fish pathogen such as *Vibrio anguillarium*, *Yersinia ruckeri*, *Pseudomonas putida* and *Aeromonas hydrophila*.

Materials and Methods

Plant and Preparation of the Extracts

The plants were purchased from herbalist in Kastamonu province. Aqueous methanolic extraction of the plants were performed as previously described (Bilen et al., 2016).

Table 1. The list of the plants used in the study

Scientific Name	Family	Vernacular Name
Laurus nobilis	Lauraceae	Laurel
Brassica nigra	Cruciferae	Black Mustard

Bacterial Strains

The plant extracts were tested against Gram negative bacteria, *Vibrio anguillarium* (SBVA1), *Yersinia ruckeri* (SBYR1), *Pseudomonas putida* (SBPP1) and *Aeromonas hydrophila* (SBAh1) which are isolated from fish and identified using conventional and molecular methods.

Minimum Inhibitory Concentration (MIC) Determination with Broth Microdilution Method

The antibacterial activity of the plants aqueous methanolic extracts were determined using sterile 300 µl 96-well plates as previously described (Wiegand et al., 2008) with small modifications. Briefly, all plant extracts were diluted up to 3200 µg ml^{-1} starting from 1.5625 µg ml^{-1} (3200 µg ml^{-1}, 1600 µg ml^{-1}, 800 µg ml^{-1}, 400 µg ml^{-1}, 200 µg ml^{-1}, 100 µg ml^{-1}, 50 µg ml^{-1}, 25 µg ml^{-1}, 12.5 µg ml^{-1}, 6.25 µg ml^{-1}, 3.125 µg ml^{-1} and 1.5625 µg ml^{-1}). 150 µl plant extract and same amount of the bacterial suspension each contains $1x10^8$ CFU was mixed by pipetting. For control, only bacterial suspension and the only methanolic extraction of the plant were prepared and added to 96-well plates. The plates were then placed in the incubator and kept at 25 °C for 48 hours. Each bacteria and all concentrations were studied in triplicate.

Results

The results of the study were given in Table 2.

Table 2. Antimicrobial activity of aqueous methanolic extracts of the medicinal plants used in the study

Plant Species	Bacterial Strains			
	VA	YR	PP	AH
Laurus nobilis (µg ml^{-1})	1600	3200	--------	3.125
Brassica nigra (µg ml^{-1})	100	3200	--------	800

VA: *Vibrio anguillarium*; YR: *Yersinia ruckeri*; PP: *Pseudomonas putida*; AH: *Aeromonas hydrophila*.

The MIC value of laurel aqueous methanolic extract showed the strongest activity against *Aeromonas hydrophila*. Black mustard aqueous methanolic extract showed the strongest activity against *Vibrio anguillarium*. Both of the plant extracts exhibit no activity against *Pseudomonas putida*. Also the activity of the plants against *Yersinia ruckeri* was very weak (3200 µg ml^{-1}).

Discussion

Medicinal herbs have many different chemical complex and substance and novel mechanism of the plans haven't been explained yet. In the present study effectiveness of the laurel and black mustard were demonstrated against *A. hydrophila* and *V. anguillarium*.

Kamaraj et al. (2012) have reported that the methanolic extract of the *A. indica* showed strong activity against *Klebsiella pneumonia*. Also, strong antimicrobial activity of *Cotinus coggyria* was determined against *Bacillus cereus*, *Bacillus subtilis*, *Staphylococcus aureus*, *Micrococcus luteus*, *Escherichia coli*, *Enterobacter aerogenes*, *Proteus vulgaris*, *Pseudomonas aeruginosa*, *Pseudomonas putida*, *Salmonella typhimurium*, *Salmonella typhi*, *Hanseniaspora guilliermondii*, *Rhodotorula rubra*, *Kluyveromyces fragilis*, *Kluyveromyces marxianus* and *Debaryomyces hansenii* (Dulger et al., 2009). Dulger et al. (2005) showed that several extracts and fractions of some Hypericum species have antimicrobial activity against bacterial pathogens.

In conclusion, this study highlights *Laurus nobilis* has antimicrobial activity against *Aeromonas hydrophila* and *Brassica nigra* has similar activity against *Vibrio anguillarium*. *In vivo* studies should be conducted using these plants extracts for the fish.

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