Published Online: 2025 May 5 **Research Article**

# The Effect of Aqueous and Ethanolic Extracts of *Nannorrhops ritchiana* Fruit on Pathogenic Bacteria *Aeromonas hydrophila*, *Yersinia rockeri* and *Streptococcus iniae* in Rainbow Trout

Mahin Rigi [1, *], Zeynab Mohkami [2]

[1] Department of Aquatic Sciences, Hamon International Wetland Research Institute, Research Institute of Zabol, Zabol, Iran
[2] Department of Agriculture and Plant Breeding, Agriculture Institute, Research Institute of Zabol, Zabol, Iran

[*] ***Corresponding Author:*** Department of Aquatic Sciences, Hamon International Wetland Research Institute, Research Institute of Zabol, Zabol, Iran. Email: mahinrigi255@gmail.com

**Received:** 3 February, 2025; **Revised:** 9 February, 2025; **Accepted:** 26 April, 2025

## Abstract

**Background:** The excessive use of antibiotics to treat bacterial diseases in aquaculture has led to drug resistance in bacterial strains, reduced the effectiveness of drugs, and caused the accumulation of antibiotics in the bodies of fish and fish consumers.

**Objectives:** The present study aimed to investigate the effect of aqueous and ethanolic extracts of the fruit of *Nannorrhops ritchiana* on the pathogenic bacteria *Aeromonas hydrophila*, *Yersinia ruckeri*, and *Streptococcus iniae* in rainbow trout.

**Methods:** Bacterial strains were prepared as standard. To determine the minimum inhibitory concentration (MIC) of aqueous and ethanolic extracts, the standard microdilution method was used, and the minimum bactericidal concentration (MBC) of the extracts was obtained based on their MIC values.

**Results:** The results of this study showed that the MIC of the ethanolic extract against *A. hydrophila*, *Y. ruckeri*, and *S. iniae* was 1.56, 6.25, and 0.78 µg/mL, respectively. In contrast, the MIC of the aqueous extract of *N. ritchiana* against these bacteria was 1.25, 25, and 6.25 µg/mL, respectively.

**Conclusions:** The results of the study demonstrated that the extract of the *N. ritchiana* plant has a very high inhibitory effect against fish pathogenic bacteria, suggesting that this plant can be used to treat and reduce infections.

*Keywords:* *Nannorrhops ritchiana*, *Aeromonas hydrophila*, *Yersinia rockeri*, *Streptococcus iniae*, Extract

## 1. Background

*Nannorrhops ritchiana*, commonly known as the Mazari palm, is locally referred to as DAAZ, wild palm, or Khok in Persian. It is recognized as the hardiest palm, with all parts of the plant being widely utilized (1). *Nannorrhops ritchiana* L. is a small, non-edible tree belonging to the Arecaceae family, distributed in tropical and subtropical regions worldwide. The fruits of this plant are berry-like and rich in oil.

Bacterial infections are a primary cause of mortality in farmed fish. The use of antibiotics and antimicrobials is the common method for treating such infections in aquaculture. However, the frequent and continuous use of antibiotics leads to the development of resistant strains in microorganisms, drug persistence in fish tissues, and environmental issues. Additionally, these chemicals inhibit the growth of bacterial flora in the digestive tract of fish (2). Due to the problem of antimicrobial resistance, there is an urgent need to establish regulations for the rational use of antibiotics and to discover new drugs and alternative treatments for controlling bacterial diseases in aquaculture (3).

Antibiotics have been identified as new pollutants in the aquatic environment, posing a serious problem due to the lack of wastewater treatment to eliminate these pollutants (4-8). Recent studies have shown that many antibiotics have entered aquatic environments, yet



**How to Cite:** Rigi M, Mohkami Z. The Effect of Aqueous and Ethanolic Extracts of *Nannorrhops ritchiana* Fruit on Pathogenic Bacteria *Aeromonas hydrophila*, *Yersinia rockeri* and *Streptococcus iniae* in Rainbow Trout. Zahedan J Res Med Sci. 2025; 27 (3): e160046. https://doi.org/10.5812/zjrms-160046.

information and findings on antibiotic interactions with aquatic organisms remain limited (9, 10).

## 2. Objectives

The present study aimed to investigate the effect of aqueous and ethanolic extracts of the fruit of *N. ritchiana* on the pathogenic bacteria *Aeromonas hydrophila*, *Yersinia ruckeri*, and *Streptococcus iniae* in rainbow trout.

## 3. Methods

### 3.1. Preparation of Methanolic and Aqueous Extracts

After collecting, washing, and drying the desired plant in the shade, it was powdered using an electric grinder. For extraction, 50 grams of the dried and ground powder of the desired part (fruit) was added to 250 mL of aqueous and ethanolic solvents and placed on a shaker for 24 hours at room temperature. The mixture was then filtered using Whatman No. 42 filter paper. After completing the extraction process, the obtained extract was concentrated using a rotary evaporator, and the extract was stored in the refrigerator until use (Figure 1).

**Figure 1.** *Nannorrhops ritchiana* fruit

### 3.2. Bacteria Preparation and Storage

*Aeromonas hydrophila* bacteria isolated from rainbow trout were prepared and approved by the Faculty of Veterinary Medicine, University of Tehran. Additionally, *Y. ruckeri* with PCR code number KEC 29653 and *S. iniae* were isolated from fish infected with Streptococcosis at the Caspian Sea Ecology Research Institute. The determination of the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of aqueous and ethanolic extracts at concentrations of 100, 50, 25, 12.5, and 6.25 mg/mL was tested using the microdilution method on the three target bacteria.

## 4. Results

The results of this study showed that the MIC of the ethanolic extracts against *A. hydrophila*, *Y. ruckeri*, and *S. iniae* were 1.56, 6.25, and 0.78 µg/mL, respectively (Table 1).

**Table 1.** Antimicrobial Effects of Ethanolic Extract of *Nannorrhops ritchiana* Fruit Plant on Pathogenic Bacteria

| Pathogenic Bacteria | 25 | 1.25 | 6.25 | 1.56 | 0.78 | 0.39 |
|---|---|---|---|---|---|---|
| *Aeromonas hydrophila* | - | - | - | + | ++ | ++ |
| *Yersinia rockeri* | - | - | + | ++ | ++ | ++ |
| *Streptococcus iniae* | - | - | - | - | + | ++ |

The results showed that the MIC of the aqueous extract of *N. ritchiana* fruit against *A. hydrophila*, *Y. ruckeri*, and *S. iniae* was 1.25, 25, and 6.25 µg/mL, respectively (Table 2).

**Table 2.** Antimicrobial Effects of Aqueous Extract of *Nannorrhops ritchiana* Fruit Plant on Pathogenic Bacteria

| Pathogenic Bacteria | 25 | 1.25 | 6.25 | 1.56 | 0.78 | 0.39 |
|---|---|---|---|---|---|---|
| *Aeromonas hydrophila* | - | + | ++ | ++ | ++ | ++ |
| *Yersinia rockeri* | + | ++ | ++ | ++ | ++ | ++ |
| *Streptococcus iniae* | - | - | - | + | ++ | ++ |

## 5. Discussion

Fish farming in ponds and fish farms is conducted in a conventional manner, which is stressful for fish species and suppresses the immune system, leading to infection and bacterial colonization. Therefore, the use of antibiotics to prevent the emergence and spread of infection is essential, especially in countries where preventive measures are not implemented. Antimicrobial drugs are widely prescribed and used in ponds and fish farms.

In a study by Beyzaei et al., the antimicrobial activity of the plant Daz was investigated, revealing the largest diameter of the inhibition zone against *S. pneumoniae* (8.40 mm), *Bacillus subtilis* (8.60 mm), and *Fusarium oxysporum* (7.66 mm) (11). The antimicrobial and antifungal activity of the ethanolic extract of this plant was tested on bacterial and fungal pathogens, showing that a concentration of 300 mg/mL had the largest

inhibitory zone diameter (5 - 21 mm) (12). Other studies also demonstrated that the ethanol extract of the aerial parts of this plant is inhibitory to the fungi *Candida albicans*, *Aspergillus niger*, and *Microsporum canis* (13).

In a study by Sutili et al., it was shown that the essential oils of *Hesperozygis ringens*, *Ocimum gratissimum*, and *O. americanum* inhibited *A. hydrophila* infections in silver catfish (14). In the study by Tafi et al., the antimicrobial effect of hydroalcoholic extracts of *Aloe vera* and sage on *S. iniae* in rainbow trout was investigated. The results indicated that the two medicinal plants, *A. vera* and *S. officinalis*, contain the active compound cineol. The MBC of *A. vera* against *S. iniae* was 4.067 mg/mL, while the MBC of *S. officinalis* was 5.185 mg/mL (15).

In the study by Fakharzadeh et al., the effect of *Origanum vulgare* extract and nano against *S. iniae* bacteria was investigated, showing that the diameter of the inhibitory zone of the extract against the bacteria was 21.7 mm, with a MIC of 0.25 mg/mL and an MBC of 0.5 mg/mL (16).

### 5.1. Conclusions

The results of the study demonstrated that this medicinal plant is an effective inhibitor of fish pathogenic bacteria and can be used in the treatment and reduction of aquatic infections.

## Acknowledgements

The authors would like to thank all the professors who contributed to the collection of data and the writing of this article.

## Footnotes

**Authors' Contribution:** Study concept and design: M. R. and Z. M. All authors read and approved the final manuscript.

**Conflict of Interests Statement:** The authors declare no conflict of interest.

**Data Availability:** The dataset presented in the study is available on request from the corresponding author during submission or after publication.

**Funding/Support:** The authors declare no financial support to write this manuscript.

## References

1. Naseem S, Naseem S, Bashir E, Shirin K, Sheikh SA. Biogeochemical evaluation ofNannorrhops ritchiana: A Mg-flora from Khuzdar, Balochistan, Pakistan. *Chinese J Geochemistry*. 2005;**24**(4):327-37. https://doi.org/10.1007/bf02873795.
2. Nasr-Eldahan S, Nabil-Adam A, Shreadah MA, Maher AM, El-Sayed Ali T. A review article on nanotechnology in aquaculture sustainability as a novel tool in fish disease control. *Aquac Int*. 2021;**29**(4):1459-80. [PubMed ID: 33688117]. [PubMed Central ID: PMC7933385]. https://doi.org/10.1007/s10499-021-00677-7.
3. Courtenay WR, Bardach JE, Ryther JH, McLarney WO. Aquaculture, the Farming and Husbandry of Freshwater and Marine Organisms. *Copeia*. 1973;**1973**(4). https://doi.org/10.2307/1443094.
4. Pal C, Bengtsson-Palme J, Kristiansson E, Larsson DG. Co-occurrence of resistance genes to antibiotics, biocides and metals reveals novel insights into their co-selection potential. *BMC Genomics*. 2015;**16**:964. [PubMed ID: 26576951]. [PubMed Central ID: PMC4650350]. https://doi.org/10.1186/s12864-015-2153-5.
5. Gogoi A, Mazumder P, Tyagi VK, Tushara Chaminda GG, An AK, Kumar M. Occurrence and fate of emerging contaminants in water environment: A review. *Groundwater Sustainable Dev*. 2018;**6**:169-80. https://doi.org/10.1016/j.gsd.2017.12.009.
6. Michael-Kordatou I, Karaolia P, Fatta-Kassinos D. The role of operating parameters and oxidative damage mechanisms of advanced chemical oxidation processes in the combat against antibiotic-resistant bacteria and resistance genes present in urban wastewater. *Water Res*. 2018;**129**:208-30. [PubMed ID: 29153875]. https://doi.org/10.1016/j.watres.2017.10.007.
7. Rodriguez-Molina D, Mang P, Schmitt H, Chifiriuc MC, Radon K, Wengenroth L. Do wastewater treatment plants increase antibiotic resistant bacteria or genes in the environment? Protocol for a systematic review. *Syst Rev*. 2019;**8**(1):304. [PubMed ID: 31806019]. [PubMed Central ID: PMC6894476]. https://doi.org/10.1186/s13643-019-1236-9.
8. Liu C, Kong D, Hsu PC, Yuan H, Lee HW, Liu Y, et al. Rapid water disinfection using vertically aligned MoS(2) nanofilms and visible light. *Nat Nanotechnol*. 2016;**11**(12):1098-104. [PubMed ID: 27525474]. https://doi.org/10.1038/nnano.2016.138.
9. Guha Roy A. Antibiotics in water. *Nature Sustainability*. 2019;**2**(5):356. https://doi.org/10.1038/s41893-019-0295-1.
10. Gomes IB, Maillard J, Simões LC, Simões M. Emerging contaminants affect the microbiome of water systems—strategies for their mitigation. *npj Clean Water*. 2020;**3**(1). https://doi.org/10.1038/s41545-020-00086-y.
11. Beyzaei H, Ebrahimnezhad Z, Dehghani M. Antioxidant and Antimicrobial Effects of Nannorrhops baluchestanica: An Endemic Palm Species in Southeast Iran. *Jentashapir J Cell Mol Biol*. 2023;**14**(1). https://doi.org/10.5812/jjcmb-134760.
12. Mahmood A. Phytochemical Analysis and Comprehensive Evaluation of Antimicrobial Activity of Nannorhops Ritchiana Leaves (Mazari Palm). *World J Pharm Pharm Sci*. 2017:173-89. https://doi.org/10.20959/wjpps20176-9336.
13. Zeeshan M. *Phytochemical and Pharmacological studies of aerial parts of Nannorrhops ritchiana [dissertation]*. Islamia University of Bahawalpur Pakistan; 2019.
14. Sutili FJ, Silva Lde L, Gressler LT, Gressler LT, Battisti EK, Heinzmann BM, et al. Plant essential oils against Aeromonas hydrophila: in vitro activity and their use in experimentally infected fish. *J Appl Microbiol*.

2015;**119**(1):47-54. [PubMed ID: 25810355]. https://doi.org/10.1111/jam.12812.

15. Tafi AA, Meshkini S, Tukmechi A, Alishahi M, Noori F. Therapeutic and Histopathological Effect of Aloe vera and Salvia officinalis Hydroethanolic Extracts against Streptococcus iniae in Rainbow Trout. *Arch Razi Inst*. 2020;**75**(2):257-87. [PubMed ID: 32621456]. [PubMed Central ID: PMC8418879]. https://doi.org/10.22092/ari.2019.122855.1232.

16. Fakharzadeh ME, Haghighi M, Sharifrouhani M, Sharifpoor I, Hamidi M. An In vitro and in vivo study on antimicrobial activity of Origanum vulgare extract and its nano form against Streptococcus iniae in rainbow trout (Oncorhynchus mykiss). *Iran J Fisheries Sci*. 2020;**19**(5):2454-63.