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The review on different species of fish's with excellent antifungal properties and its various extraction methods

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Abstract
Aquaculture, veterinary medicine, and human health are all seriously threatened by fungal infections. The development of new, environmentally safe, and sustainable treatments is required due to the growing worry over antifungal resistance. The antifungal qualities of several fish species, such as tilapia, rainbow trout, Sphyraena putnamae Channa, striata, winter flounder, are examined in this study. Significant antifungal action against Candida, Aspergillus, Fusarium, and Saprolegnia was shown by fish skin mucus and extracts. Strong antifungal effects were demonstrated by bioactive chemicals extracted from fish, indicating their potential as natural antifungal agents. This study highlights the potential of antifungals produced from fish in the treatment of fungal infections, providing a novel strategy to tackle the worldwide health issue of fungal diseases.

Keywords: Tilaphia, rainbow trout, Channa striata, winter flounder, Sphyraena putnamae, candida albican, aspergillus, saprolegnia.

Introduction
For many individuals, fish is an essential part of their diet and frequently provide the nutrients they need to live a healthy life. Compared to other protein-rich foods, fish is a highly affordable source of dietary protein. [1] According to research fish is known to have exceptionally high-quality lipids and oil, and fish oil has a high content of polyunsaturated fatty acids, which are crucial for decreasing blood cholesterol levels. [2] Conversely, fat-soluble vitamins are present in fish oil. Fishes have long been used to treat infections, ease pain, and treat patients recovering from surgeries, wounds, and injuries. Both freshwater and marine fish, as well as shellfish, contain nutrients that are necessary for human growth and development, including thiamine and riboflavin, as well as minerals, phospholipids, sterols, enzymes, hormones, hydrocarbons, and pigments. [3] The muscle of fish contains four basic nutrients in different amounts: water 70–80%, protein 16–25%, lipids 1% to 5%, and vitamins. [4] Under typical circumstances, the fish's intricate network of innate defense systems allows them to protect themselves from these possible invaders. [5] Fish's innate immune system is separated into three parts: humoral, cellular (phagocytic cells and nonspecific cytotoxic cells), and physical (scales on the body and cutaneous mucus surfaces of skin, gills, and guts), which serve as the body's first line of defense against infection. [6] In recent decades, fungal infections have become much more common and severe, with high rates of morbidity and death, particularly in individuals with impaired immune systems. According to estimates, fungal illnesses kill at least 1.5 million people annually and impact around 1.2 billion people globally. Additionally, the extensive use of antifungal medications is having detrimental effects on antifungal therapy as resistant strains are emerging, which results in treatment of fungal infection [7]

Materials and Methods
Comprehensive insights about fish
1. Rainbow trout
2. Sphyraena putnamae
3. Winter Flounder

1. Rainbow trout [8, 9, 10]

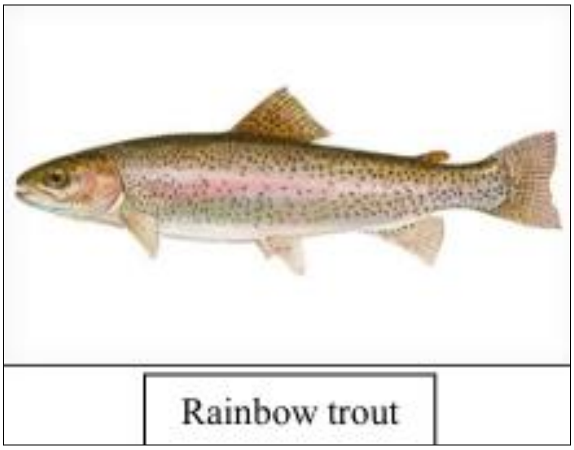

Fig 1: Rainbow trout

Kingdom: Animalia
Phylum: Chordata
Class: Actinopterygii
Order: Salmoniformes
Family: Salmnidae
Genus: Oncorhynchus
Rainbow trout
Species: O. mykiss

Chemical Constituents
Proteins (20%): Essential amino acids, peptides and enzymes, Fatty Acids (10-15%): Omega-3 fatty acids (EPA, DHA), omega-6 fatty acids, and saturated fats, Vitamins: B12, B6, Antioxidants: Astaxanthin.

Uses
Fish oil-derived omega-3 supplements for cardiovascular health.
Astaxanthin-based antioxidants in skincare and supplements and antifungal effects. Collagen and gelatin extracts for cosmetic and pharmaceutical applications.

2. Sphyraena putnamae [11, 12]

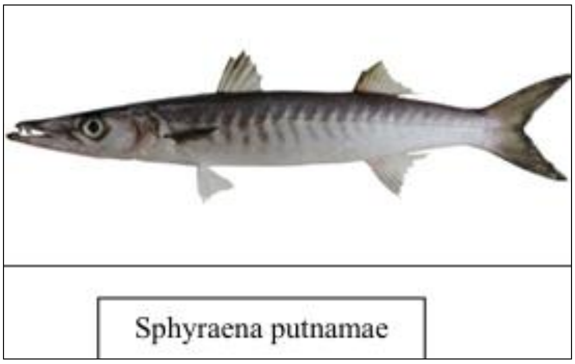

Fig 2: Sphyraena putname

Kingdom: Animalia
Phylum: Chordata
Class: Actinopterygii
Order: Perciformes
Family: Sphyraenidae
Genus: Sphyraena
Species: S. putnamae.

Chemical Constituents
Proteins (20-25%): riboflavin, and thiamin, Minerals: Phosphorus, potassium, sodium, calcium, magnesium, and iron, Antioxidants: vitamin E, and selenium.

Uses
Selenium. based antioxidants in skincare and supplements. Collagen and gelatin extracts for cosmetic and pharmaceutical applications and use as antimicrobial agent.

3. Winter Flounder [13]

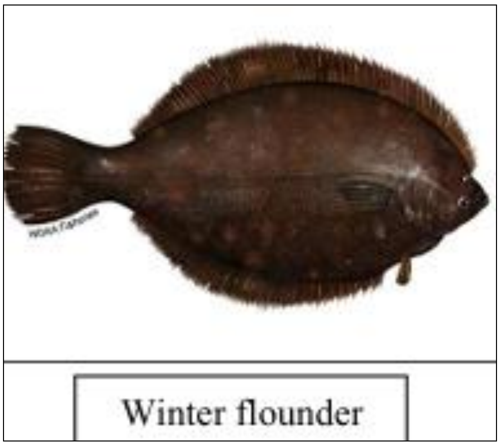

Fig 3: Winter Flounder

Kingdom: Animalia
Phylum: Chordata
Class: Actinopterygii
Order: Pleuronectiformes
Family: Pleuronectidae
Genus: Pseudopleuronectes
Species: P. americanus

Chemical Constituents
Proteins (18%): (EPA, DHA), Bioactive compounds: - Glycoproteins: Exhibiting antimicrobial and antiviral activities. - Fish-derived collagen: Used in cosmetic and pharmaceutical applications.

Uses
Fish-derived collagen: Used in wound healing, skin care, and joint health products. Antimicrobial peptides: Derived from Winter Flounder for wound healing and infection control.

Extraction methods: [14]

1. Extraction of collgen from fish
The skins were promptly frozen in a double drum spiral freezer, transported to the laboratory in refrigerator, and then kept at 20 degrees Celsius. The skins were thawed in room temperature tap water before being cut with scissors into pieces of around 5 × 5 mm. To get rid of fat and other residues, 50 g of skin pieces were washed with ultrapure water at room temperature for 30 minutes and then combined with two volumes (v/w) of ethanol for 24 hours at room temperature without stirring. Following three rounds of ultrapure water washing, the skin pieces were placed aside till the water was drained away.

2. Sodium hydroxide method:
1. The sodium hydroxide method (1000 mL 0.12 M NaOH for 24 hours at room temperature),
2. The acetic acid method (1000 mL 0.5 M acetic acid for 24 hours at room temperature),
3. For the sodium hydroxide and acetic acid methods, this step was completed at 4C.

3. Hot water method
The hot water method (1000 mL 80c ultra pure water for 6 hours in an oscillatory water bath shaker incubator 10 rpm). A second laer of gauze was then used to filter the samples. Dialysis cassettes were used to dialyze the resulting solution in ultrapure water for 72 hours. For the hot water approach, it was completed at room temperature. In order to obtain pure dried collagen, pure collagen solutions were finally obtained and vacuum freeze dried. A digital camera was used to take pictures of the collagen samples that were collected.

4. Extraction of oil from fish
Three techniques were used to extract oil samples:

a. Microwave-assisted extraction
Hexane was the ideal solvent, the solid-liquid ratio was 1:10 (m/v). The microwave power was 100 W. The extraction period was 10 minutes for the microwave-assisted technique. It is centrifuged at 8000 rpm extraction. Prior to examination, the oil samples were kept at -80°C after being condensed on 0.22 μm filters. [15]

b. Dilute alkali hydrolysis
Dilute alkali hydrolysis is accomplished by placing several freeze-dried tilapia samples in a triangle bottle, adding the appropriate quantity of deionized water, and adjusting the

solid-liquid ratio (1:2.5, m/v). using a 10 g/100 mL KOH solution to get the pH down to 8.0. Conditions for hydrolysis: 1576 X. LI *ET AL*., 55°C, 30 minutes, adding 3 g of KNO3 per 100 g of raw materials. Centrifuging at 8000 r/min; and siphoning out the top liquid.

c. Ezymatic hydrolysis

Enzymatic hydrolysis is processed placing freeze-dried tilapia samples in a triangle bottle. Adding the appropriate volume of deionized water and adjusting the solid-liquid ratio (1:2, m/v). Using a 10 g/100 mL KOH solution to get the pH down to 9.0, Hydrolysis conditions: 0.8 g/100 g enzyme dose, 55°C, and 2.5 h. Enzyme deactivation: 90°C for 5 minutes, centrifugation at 8000 rpm, and syphoning off the top liquid.

Extraction of gelatin from fish [16]

After being moved to the lab, the samples were put in an ice box. The knife was used to remove the scales, after which they were cleaned and ready for extraction.

Gelatin enzymatic extraction

Gelatin was enzymatically extracted from fish scales using NaOH and HCL to eliminate non-collagen protein components. After that, the scales were immersed in 1:10 w/v pure water (pH: 6). A 0.01% quantity of the bromelain enzyme (Sigma) was added. incubated for two hours at 45°C in a water bath. After dampening the enzyme action for five minutes in a hot water bath, the mixture was filtered through cheese cloth and What Man filter paper. [16]

Gelatin thermal extraction

After washing 14.3 g of scales with tap water for an hour. They were rinsed with 100 ml of 0.4 w/v NaOH for four hours to remove non-collagen protein substances. The scales were then rinsed with tap water until they were neutralized, and then they were submerged in 100 ml of 0.4 v/v HCL for four hours before being rinsed with tap water again until they were neutralized. The scales were heated to 70°C for one and a half hours after being submerged in distilled water at a ratio of (1:1) g/mL. After using cheesecloth to remove the solution from the scales, the solution was concentrated for 30 minutes using a rotary evaporator and dried for 18 hours at 50°C. Gelatin was kept in a freezer until the test process. [16]

Conclusion

To sum up, our study shows that several fish species with strong antifungal qualities against [candida albican] include rainbow trout, sphyraena putnamae, channa striata, winter flounder, and tilaphia insert. These fish species include bioactive substances such chhanine, glycoprotein, fish-derived collagen, and protein hydrolysates that have the potential to be sustainable and natural treatments for fungal infections. The study's findings emphasize how crucial it is to investigate marine resources in order to find new antifungal medicines. Additionally, these fish species' antifungal activity points to possible uses in the creation of novel therapies for illnesses linked to fungi that affect both people and animals. Furthermore, compared to synthetic chemicals, the usage of antifungals produced from fish may offer a more sustainable and eco-friendly option. *In vivo* studies to assess the safety and effectiveness of these compounds in treating fungal infections should be the main focus of future research, along with the isolation and characterization of the bioactive molecules causing the antifungal action. All things considered, this study shows that fish species have the potential to be a useful source of antifungal drugs and merits more research.

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