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# EVALUATING THE ANTIMICROBIAL POTENTIAL OF *MYRIOPHYLLUM SPICATUM* (EURASIAN WATERMILFOIL) AGAINST SOME FISH PATHOGENS IN THE COLD WATERS OF THE KASHMIR HIMALAYA

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## ABSTRACT

**The use of antibiotics in the aquaculture leads to antibiotic-resistant aquatic pathogens. Plant-derived medications are gaining attraction due to their perceived safety and efficacy. Medicinal plants show promise in combatting infections and minimizing adverse effects. Aquatic weeds produce compounds effective against bacterial pathogens. India utilizes traditional medicinal plants, and seaweed exploration offers potential for therapeutic drug development. Extracting active compounds from aquatic plants can uncover their antimicrobial potential. The study aims to evaluate antimicrobial potential in *Myriophyllum spicatum* extracts against fish bacterial pathogens, using various solvents. Methanolic extract exhibits the highest antibacterial activity, emphasizing the effect of various solvents against different fish diseases. Natural hydrophytic plant extracts are gaining interest as antimicrobials to combat foodborne diseases and reduce reliance on synthetic drugs. *Myriophyllum spicatum* exhibits potential as a natural remedy for aquaculture infectious diseases, showing promise against a spectrum of bacteria and aquaculture-related pathogens.**

**KEY WORDS :** Aquaculture, Aquatic weeds, Fish farming's, Hydrophytic plant, *Myriophyllum spicatum*

## INTRODUCTION

Plants, herbs, and ethnobotanicals have been utilized since ancient times and remain globally important for promoting health and treating diseases (Ertürk, 2017). Medicinal and aromatic plants have a rich history of use, not only in traditional medicine but also in various industries like spices, pharmaceuticals, and cosmetics. Interestingly, many synthetic drugs share similarities with active components isolated from plants. About a quarter of the active constituents in pharmacologically produced drugs are sourced from plants (WHO, 2013). In recent times, there has been a notable rise in the demand for medicinal plants. According to the World Health Organization (WHO), approximately 80% of the population in developing countries predominantly relies on plant-based traditional medicine to address their essential health needs. *Myriophyllum spicatum*, also recognized as Eurasian watermilfoil, is a perennial submerged aquatic plant primarily reproducing through vegetative fragmentation (Aiken *et al.*, 1979). These fragments are generated throughout

the year, often with roots developing on a fragment before the plant detaches. The plant can thrive in water depths ranging from 0.5 to 10 meters, although it is most commonly found in waters from 0.5 to 3.5 meters deep. Rooted to the bottom, it grows towards the water's surface, extensively branching out upon reaching the surface to create a dense canopy. Flowering and seed production are frequent events. India holds the second position globally in total fish production, with an annual fisheries yield of around 9.06 million metric tonnes. In the European Union, farm animals consume a significant 4,700 tonnes of antibiotics, constituting 35 percent of the total, while human consumption accounts for 65 percent or 8,500 tonnes. Among antibiotics administered to animals, 3,900 tonnes (29 percent of the total) aid ailing animals in their recovery, while 786 tonnes (6 percent of the total) enhance farm animal growth. A survey estimated a 50 percent reduction in antibiotic use for growth since 1997, when animals consumed approximately 1,600 tonnes as feed additives (FEDESA). The widespread use of antibiotics in fish farming has been linked to antibiotic resistance in various aquatic pathogens, including *Aeromonas salmonicida, A. hydrophila, Edwardsiella icttaluri, E. tarda, Vibrio salmonicida, V. anguillarum, Yersinia ruckeri,* and *Pasteurella piscida* (De Paola *et al.,* 1995). Immediate and rigorous controlled studies are imperative to comprehensively evaluate the repercussions of antimicrobial therapy on the microorganism ecosystem within aquaculture ponds. The enthusiasm for plant-derived medications is growing, primarily due to the prevailing belief that "green medicine" is not only safer but also more reliable compared to synthetic drugs, many of which carry adverse side effects. The continual use of antimicrobial agents in aquaculture has led to the development of more resilient bacterial strains in aquatic environments. Prolonged reliance on synthetic antibiotics poses a looming threat to consumers and non-target organisms in the environment (Abutbul *et al.*, 2005). The treatment of bacterial ailments with diverse herbs has been safely employed in organic agriculture, veterinary medicine, and human healthcare (Direkbusarakom, 2004). Since ancient times, medicinal plants have played a pivotal role in treating common infectious diseases (Rios and Recio, 2005), and utilizing plants with antibacterial properties shows great promise as an alternative approach in aquaculture (Abutbul *et al.,* 2005). Medicinal plants offer effective means to combat infectious diseases while mitigating many of the adverse effects of synthetic antimicrobials (Punitha *et al.,* 2008). *Aeromonas hydrophila*, the prevalent bacterial pathogen in freshwater fish, is known to be responsible for various pathological conditions, comprising tail/fin-rot and hemorrhagic septicemia, particularly in freshwater and ornamental fish (Austin and Austin, 2007). Seaweeds inhabiting the depths of the ocean face a continuous threat from potentially dangerous co-existing microbes. To counter this, they have developed sophisticated chemical defense mechanisms by producing a diverse range of secondary metabolites (Kubanek *et al.,* 2003; Kolanjinathan *et al.,* 2009). These compounds, including fatty acids, alkaloids, glycosides, flavonoids, saponins, tannins, and steroids found in seaweeds, have exhibited notable effectiveness against various bacterial pathogens affecting humans and fish (Bansemir *et al.,* 2006), plant leaf spot diseases (Kumar *et al.,* 2008), and marine pathogenic microorganisms (Engel *et al.,* 2006). In India, a diverse range of medicinal plants has been traditionally harnessed to address a myriad of ailments. The exploration of bioactive substances within seaweeds presents a promising avenue for the development of novel therapeutic drugs. Extensive global research has focused on marine algae, resulting in the isolation of life-saving drugs and biologically active compounds (Martinez-Nadal *et al.,* 1963; Caccamese, *et al.,* 1980). Reports highlight that more than 15,000 marine natural products were successfully isolated between 1965 and 2005 (Blunt *et al.,* 2007). In this study, a variety of organic solvents have been employed to extract potential active compounds from aquatic plants. The primary objective is to uncover the antimicrobial activity exhibited by these selected aquatic plant extracts against bacteria and fungi. However, there remains a dearth of knowledge concerning the antimicrobial potential of hydrophytes from Kashmir, particularly as a natural remedy for fish bacterial pathogens. Thus, this investigation aims to evaluate the antibacterial potential of ethanol, acetone, methanol, petroleum ether, and water extracts obtained from the submersed aquatic plant *Myriophyllum spicatum* (Eurasian watermilfoil), a member of the Haloragaceae family. This research intends to shed light on the effectiveness of these extracts against specific clinical isolates of fish bacterial pathogens, paving the way for potential applications in aquaculture and related fields.

## MATERIALS AND METHODS

### Collection and Identification of Plant Material

The aquatic plant species, *Myriophyllum spicatum*, was obtained from Dal Lake in Kashmir, India. Specimens were retained as herbarium samples at the Division of Aquatic Environment Management (AEM), SKUAST-Kashmir, Faculty of Fisheries.

### Preparation of Extracts

Approximately 1044 grams of *Myriophyllum spicatum*, as a whole plant sample, were collected and subjected to shade drying at a controlled temperature of 30±2 °C. After drying, the plant material was finely ground into a coarse powder using a grinder. Subsequently, five distinct extracts were prepared from *Myriophyllum spicatum* using different solvents: ethanol, petroleum ether, acetone, methanol, and water.

### Petroleum Ether Extract

To obtain petroleum ether extracts, 50 g of *Myriophyllum spicatum* powder were placed in a thimble and subjected to extraction in a Soxhlet apparatus using 250 ml of petroleum ether. The temperature was maintained between 50-60 ºC throughout the process. The resulting dark-colored extract was collected and air-dried for 24 hours before being stored in McCartney bottles at 4 ºC. This extraction process was repeated three times to ensure an ample supply of extracts.

### Acetonic Extract

For acetonic extracts, 50 g of *Myriophyllum spicatum* powder were packed into a thimble and extracted in a Soxhlet apparatus using 250 ml of acetone. The temperature was maintained between 45-55ºC during the 4-hour extraction process. The dark-colored extract was collected, air-dried for 24 hours, and stored in McCartney bottles at 4ºC. This process was repeated three times.

### Ethanoic Extract

Ethanoic extracts were prepared by subjecting 50 grams of *Myriophyllum spicatum* powder to extraction in a Soxhlet apparatus with 250 ml of ethanol. The temperature was maintained between 60-80 ºC during the 4-hour extraction process. The dark-colored extract was collected, air-dried, and stored in McCartney bottles at 4 ºC. This process was repeated three times.

### Methanolic Extract

Methanolic extracts were prepared in a manner similar to ethanolic extracts but using methanol as the extraction solvent. The dried extracts were collected and stored in McCartney bottles after overnight drying.

### Aqueous Extract

A total of 40 g of *Myriophyllum spicatum* powder were combined with 280 ml of distilled water. After two days, the liquid extract was filtered, and the filtrate was dried in a water bath at a temperature range of 80-90 ºC. The resulting dried extracts were stored in McCartney bottles at 4 ºC.

### Storage of Extracts

All extracts were collected in 25 ml McCartney bottles and dried until they had a sticky, glutinous appearance. Various quantities of extracts were obtained, and the bottles were sealed and stored in the refrigerator at 4 ºC.

### Preparation of Stock Solutions for Phytochemical Assays

For phytochemical assays, the extracts were dissolved to create a stock solution of 5 µg/l by combining 0.5 g of crude extract with 100 ml of solvent, ensuring consistency in preparing all five stock solutions.

### Biochemical Assays

A preliminary screening of the extracts was conducted using biochemical tests to identify various phytochemicals in *Myriophyllum spicatum*. The extracts were tested for the presence or absence of secondary metabolites, including alkaloids, phenolic compounds, steroidal compounds, saponins, tannins, flavonoids, and cardiac glycosides, following the methods outlined by Harborne (Direkbusarakom, 2004).

### Antibacterial Activity Test

The well diffusion method was used to assess the antibacterial characteristics of the samples, with both positive and negative controls included for comparison.

### Test Organisms

Bacterial strains used in the experiment were obtained from the Institute of Microbial Technology's (IMTECH) Microbial Type Culture Collection in Chandigarh, India.

**Bacterial Strains Obtained from IMTECH**

a. *Bacillus subtilis*
b. *Escherichia coli*
c. *Staphylococcus aureus*
d. *Pseudomonas aeruginosa*
e. *Aeromonas hydrophila*

**Preparation of Extract Solutions for Antibacterial Activity Test**

To perform the antibacterial activity test, all five types of dried *Myriophyllum spicatum* extracts were dissolved in 10% DMSO. This resulted in the creation of five different concentrations of extract solutions. Specifically, 4 g of extract were dissolved in 50 ml of 10% DMSO to prepare a solution with a concentration of 100 µg/µl. Subsequently, this solution was diluted to create concentrations of 80 µg/µl, 50 µg/µl, 30 µg/µl, and 10 µg/µl. For all inoculations during the test, a fresh stock solution of the extract was accurately prepared.

**Media Preparation**

The composition of the nutrient agar is outlined in Table 1. For the antibacterial activity test, Muller Hinton Agar (MHA) was used, and its composition can be found in Table 2. Fresh cultures were prepared using Nutrient Agar (NA) medium.

**Preparation of Fresh Nutrient Agar**

In order to make a sufficient amount of this medium, we accurately measured each ingredient and placed them in a conical flask. We added distilled water to reach the desired volume. The mixture was then heated on a Bunsen burner until the solution became clear. Once it was clear, the medium was autoclaved for 1.5 hours to ensure it was completely sterile and free of any impurities. After autoclaving, we immediately poured the medium into plates to prevent it from solidifying. The plates we used could hold 20 ml of medium and were labeled appropriately before we poured the medium into them. Once the plates were filled, we allowed them to solidify in the laminar airflow and then stored them in the refrigerator for future use.

**Table 1.** Agar's nutritional composition

| Ingredients | Amount |
|---|---|
| Peptone | 0.5% |
| Beef extract/Yeast extract | 0.3% |
| Agar | 1.5% |
| NaCl | 0.5% |
| Distilled Water | 20 ml |
| Final pH | 7.4 ± 0.2 |

**Table 2.** Muller Hinton Agar composition

| Ingredients | Amount |
|---|---|
| Beef infusion form | 30% |
| Caesinhydrolysate | 1.75% |
| Starch | 0.15% |
| Agar | 1.7% |
| pH | Neutral |

**Preparation of Stock Cultures**

In the antibacterial assay, we used five bacterial strains. Two of these were Gram-positive bacteria: *Staphylococcus aureus* (MTCC-2940) and *Bacillus subtilis* (MTCC-441). Additionally, we employed three Gram-negative bacteria: *Escherichia coli* (MTCC-739), *Pseudomonas aeruginosa* (MTCC-424), and *Aeromonas hydrophila* (ATCC-7966). We sourced these bacterial strains from the Microbial Type Culture Collection at the Institute of Microbial Technology (IMTECH) in Chandigarh, India. To maintain these bacterial strains, we sub-cultured them on Muller Hinton Agar (Himedia) every fifteen days and stored the cultures at 4 ºC. For the antibacterial assay, we used gentamycin discs from EOS Laboratories, India, as positive controls and 10% Dimethylsulfoxide (DMSO) as the negative control.

In a sterile environment, we sub-cultured the organisms on freshly prepared Nutrient Agar (NA) plates using a sterile loop, starting from pure cultures. The pure cultures, which we obtained from the departmental stock refrigerator, were thawed in the incubator for 30 minutes. Using a metal loop in the laminar airflow, we streaked the organisms onto the freshly prepared nutrient agar plates. We

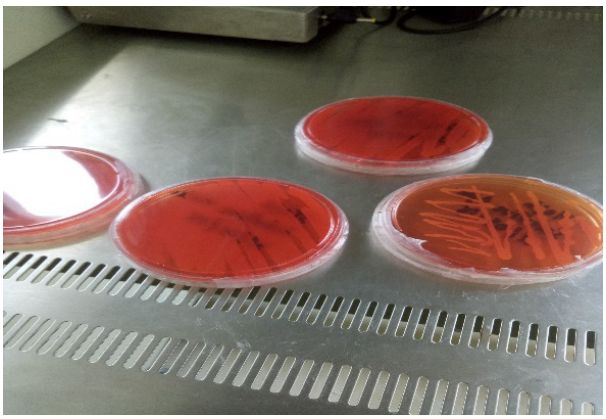

**Fig. 1.** Fresh cultures for antibacterial tests

properly labeled these inoculated plates and incubated them at 37 ºC for 24 hours to ensure optimal growth. We then used the resulting fresh cultures for the antibacterial tests (Figure 1).

**Preparation of Test Plates**

To perform the biochemical tests, we used Muller Hinton agar (MHA). We began by mixing 2.1 grams of Muller Hinton agar with 100 ml of distilled water. This mixture was stirred carefully with a sterile spatula, heated to ensure complete dissolution of the medium, and then autoclaved for 1.5 hours to sterilize it. Subsequently, we poured 20 ml of the prepared medium into sterile plates, labeled them appropriately, and allowed them to solidify.

**Inoculation of Test Organisms**

To create a cell suspension, the test organisms were transferred with a loop into test tubes containing 5 ml of 0.9% saline. These organisms were vortexed in a vortex machine to ensure thorough mixing in the saline solution. A 0.5 McFarland turbidity standard, equivalent to 108 cfu/ml of inoculum, was manually prepared by adding 0.5 ml of 0.048 M $BaCl2.2H_2O$ to 99.5 ml of 1% $H_2SO_4$, with continuous stirring to maintain a suspension whose density was confirmed by measuring absorbance spectrophotometrically at 625 nm. The absorbance fell within the range of 0.08 to 0.13. The turbidity of this standard was then compared to that of the bacterial inoculums. If the bacterial inoculums appeared more turbid than the 0.5 McFarland standard, additional broth was added to achieve the desired turbidity. The bacteria from the cell suspension were then promptly introduced into the freshly prepared MHA medium using a cotton swab, ensuring a uniform distribution of organisms in the medium by rotating the swab 90 degrees each time.

**Placement of Extracts and Controls in the Plates**

The plates, which contained the inoculated samples, were appropriately labeled. Using a cork borer, we created wells in the agar. Each well received 100 µl of a stock extract, which had been prepared in advance at concentrations of 100 µg/ml, 80 µg/ml, 50 µg/ml, 30 µg/ml, and 10 µg/ml using freshly prepared 10% dimethyl sulfoxide (DMSO). A positive control was established using Gentamycin against all bacteria, while a negative control employed 10% freshly prepared DMSO in one of the wells (Figure 2). The plates were then labeled and incubated at 37 °C for 24 hours.

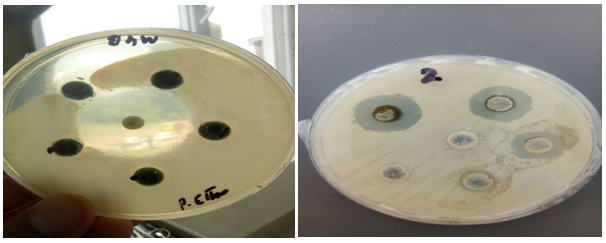

**Fig. 2.** Positive (Gentamycin) and negative (DMSO) controls for comparison of AST results

**Measuring Zone**

Following a 24-hour incubation period for the test plates, we measured the clear zones using a ruler. This measurement included determining the entire diameter of the transparent zone, and we carefully recorded these measurements. The minimum inhibitory concentration (MIC) was determined as the lowest concentration of the extract, ranging from 10 to 100 mg/ml, that successfully inhibited the growth of the test microorganisms.

## RESULTS

**Phytochemical analysis**

Phytochemical analysis of the hydrophyte revealed the presence of various secondary metabolites. Cardiac glycosides, flavonoids, phenols, steroids and tannins were detected in both the aqueous and methanolic extract. Anthraquinones, Glycosides, cardenolides, steroids, terpenoids, phlobtannins and volatile oils were found to be absent in both the extracts as shown in Table 3.

**Antibacterial activity**

The methanolic extract showed maximum activity against all the tested bacterial strains with the zone of inhibition equal to 16 mm against *Pseudomonas aeruginosa*, 19 mm against *Escherchia coli*, 16 mm against *Staphylococcus aureus,* 16 mm against *Bacillus subtilis*and 14 mm against *Aeromonas hydrophilla* at the concentration of 100 mg/ml. The acetone extract also showed considerable activity with the zones of inhibition equal to 15 mm against *Escherchia coli* at the concentration of 100 mg/ml. 10% DMSO (negative control), showed no activity against any of the tested bacterial strains. The results were compared to positive control (Gentamycin), which showed the zone of inhibition of 25 mm against *Bacillus subtilis* and *Pseudomonas aeruginosa,* 26 mm

**Table 3.** Preliminary phytochemical screening of petroleum ether, acetone, ethanol, methanol and water extracts of *Myriophyllum spicatum*

| S. No. | Phytochemicals | Petroleumether | Acetone | Ethanol | Methanol | water |
|---|---|---|---|---|---|---|
| 1 | Alkaloids | - | + | + | + | - |
| 2 | Anthraquinones | - | - | - | - | - |
| 3 | Glycosides | - | - | - | - | - |
| 4 | Cardenolides | - | - | - | - | - |
| 5 | Saponins | - | + | - | - | + |
| 6 | Steroids | - | - | - | - | - |
| 7 | Tannins | - | - | - | - | - |
| 8 | Phlobtannins | - | - | - | - | - |
| 9 | Flavonoids | - | - | + | + | - |
| 10 | Terpenoids | - | - | - | - | - |
| 11 | Phenols | - | + | + | + | + |
| 12 | Volatile oils | - | - | - | - | - |

Note: + (present), & - (absent)

against *Staphylococcus aureus*, 20 mm against *Escherichia coli* and 25 mm against *Aeromonas hydrophilla* (Table 4 and Figure 3).

**Minimum inhibitory concentration (MIC)**

MIC of the methanolic extract was 50 mg/ml against *Staphylococcus aureus* in both the hydrophyes, *Myriophyllum spicatum* (Table 5) as well as in *Myriophyllum spicatum* (Table 5). As far as methanolic extract is concerned, MIC was 30 mg/ml, but was undetected in rest of the test microbes.

## DISCUSSION

The emergence of treatment resistance among aquaculture diseases, as well as the negative effects of certain antibacterial and antifungal drugs, has

**Table 4.** Zones of inhibition (in mm) of aqueous, petroleum ether, acetone, ethanol, methanolic extract of *Myriophyllum spicatum* against bacterial strains

| S. No. | Bacterial strain | Solvent | Concentration of *M. spicatum* extract (mg/ml) | | | | | Gentamycin (10 µg/disc) |
|---|---|---|---|---|---|---|---|---|
| | | | 10 | 30 | 50 | 80 | 100 | |
| 1 | *Staphylococcus aureus* | Petroleum ether | - | - | - | - | - | 26.20±0.00 |
| | | Acetone | - | - | - | - | - | 26.20±0.00 |
| | | Ethanol | - | - | - | - | - | 26.20±0.00 |
| | | Methanol | - | - | $12.68^{a}$±0.82 | $13.47^{a}$±0.56 | $16.28^{b}$±0.71 | $26.20^{c}$±0.00 |
| | | Water | - | - | - | - | - | 26.20±0.00 |
| 2 | *Bacillus subtilis* | Petroleum ether | - | - | - | - | - | 25.22±0.00 |
| | | Acetone | - | - | - | - | - | 25.22±0.00 |
| | | Ethanol | - | - | - | - | - | 25.22±0.00 |
| | | Methanol | - | $11.73^{a}$±0.84 | $12.81^{b}$±0.53 | $13.88^{c}$±0.25 | $16.43^{d}$±0.52 | $25.22^{e}$±0.00 |
| | | Water | - | - | - | - | - | 25.22±0.00 |
| 3 | *Escherichia coli* | Petroleum ether | - | - | - | - | - | 20.20±0.00 |
| | | Acetone | - | $11.59^{a}$±0.57 | $12.59^{b}$±0.49 | $14.54^{c}$±0.29 | $15.23^{d}$±0.15 | $20.20^{e}$±0.00 |
| | | Ethanol | - | - | - | - | - | 20.20±0.00 |
| | | Methanol | - | $14.55^{a}$±0.48 | $16.13^{b}$±0.20 | $18.37^{c}$±0.19 | $19.15^{d}$±0.21 | $20.20^{e}$±0.00 |
| | | water | - | - | - | - | - | 20.20±0.00 |
| 4 | *Pseudomonas aeruginosa* | Petroleum ether | - | - | - | - | - | 22.86±5.77 |
| | | Acetone | - | - | - | - | - | 22.86±5.77 |
| | | Ethanol | - | - | - | - | - | 22.86±5.77 |
| | | Methanol | - | $12.01^{a}$±0.69 | $14.43^{a}$±0.74 | $15.24^{a}$±0.24 | $16.01^{a}$±0.59 | $22.86^{b}$±5.77 |
| | | water | - | - | - | - | - | 22.86±5.77 |
| 5 | *Aeromonas-hydrophilla* | Petroleum ether | - | - | - | - | - | 25.02±0.00 |
| | | Acetone | - | - | - | - | - | 25.02±0.00 |
| | | Ethanol | - | - | - | - | - | 25.02±0.00 |
| | | Methanol | - | - | $10.50^{a}$±o.36 | $12.09^{b}$±0.82 | $13.99^{c}$±0.67 | $25.02^{d}$±0.00 |
| | | water | - | - | - | - | - | 25.02±0.00 |

Mean ± SE values with different superscripts row-wise differ significantly ($p \leq 0.05$)

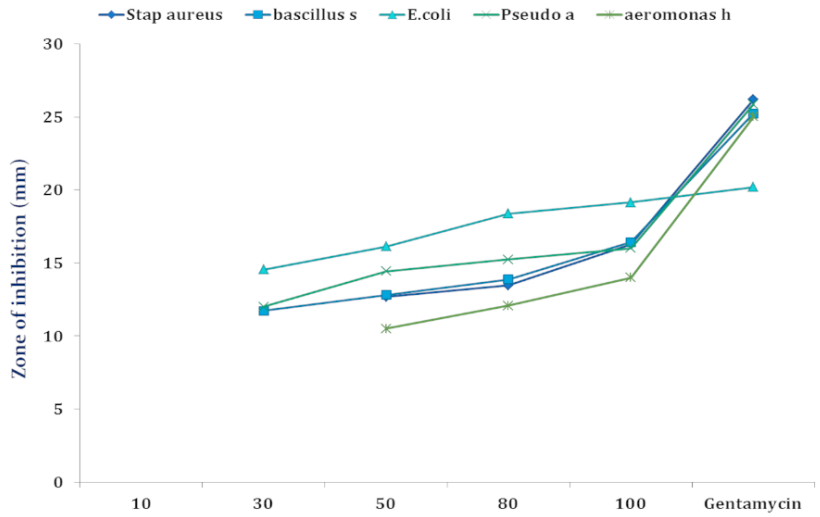


**Fig. 3.** Zones of inhibition (in millimeter) of *Myriophyllum spicatum* extracts against bacterial strains

aroused serious concerns. As a result, novel antimicrobial drugs are desperately needed (Phongpaichit *et al.,* 2005). The growing resistance of germs to antibiotics poses a serious global concern. This rise in bacterial resistance has reignited interest in the antibacterial capabilities of herbs against resistant strains (Hemaiswarya *et al.,* 2008; Alviano and Alviano, 2009). Many plant leaves possess antimicrobial properties attributed to compounds such as tannins, essential oils, and various aromatic substances. Moreover, plant tannins and flavonoids have demonstrated noteworthy biological activities and antibacterial properties. Plant defense mechanisms against invading pathogens and various environmental stresses heavily rely on phenolic chemicals (Hellio *et al.,* 2001). Additionally, phenolic compounds and their derivatives, including flavonoids, tannins, phenyl propanoids, simple phenols, lignins, and other substances, contain aromatic rings and hydroxyl groups that influence the compound's radical scavenging capacity (Dziedzic *et al.,* 1983). The present study focused on extracting hydrophyte *Myriophyllum spicatum* using five distinct solvents (petroleum ether, methanol, acetone, ethanol, and water). Biochemical assays were conducted to confirm the presence of several phytochemicals, encompassing alkaloids, phenols, flavonoids, tannins, steroids, saponins, and cardiac glycosides. The analysis of phytochemicals revealed a diverse array of secondary metabolites. Both the aqueous and methanolic extracts showed the presence of cardiac glycosides, flavonoids, phenols, steroids, and tannins. However, terpenoids, anthraquinones, cardenolides, glycosides, steroids, phlobtannins, and volatile oils were not detected in either of the

**Table 5.** Minimum inhibitory concentration (MIC) of aqueous and petroleum ether, acetone, ethanol, methanolic extracts of *Myriophyllum spicatum* against the tested bacterial strains.

| S. No. | Bacterial strain | Solvent | MIC (mg/ml) |
|---|---|---|---|
| 1 | *Escherichia coli* (MTCC-739) | Petroleum ether | ND |
| | | Acetone | 30 |
| | | Ethanol | ND |
| | | Methanol | 30 |
| | | Aqueous | ND |
| 2 | *Staphylococcus aureus* (MTCC-2490) | Petroleum ether | ND |
| | | Acetone | ND |
| | | Ethanol | ND |
| | | Methanol | 50 |
| | | Aqueous | ND |
| 3 | *Pseudomonas aeruginosa* (MTCC-2940) | Petroleum ether | ND |
| | | Acetone | ND |
| | | Ethanol | ND |
| | | Methanol | 30 |
| | | Aqueous | ND |
| 4 | *Bacillus subtilis* (MTCC-441) | Petroleum ether | ND |
| | | Acetone | ND |
| | | Ethanol | ND |
| | | Methanol | 30 |
| | | Aqueous | ND |
| 5 | *Aeromonas hydrophilla* (ATCC- 7966) | Petroleum ether | ND |
| | | Acetone | ND |
| | | Ethanol | ND |
| | | Methanol | 30 |
| | | Aqueous | ND |

ND = not detected within the observed range (10-100 mg/ml).

extracts. These findings align with the results reported by (Belkacem *et al.,* 2014) during their phytochemical screening of various *Punicagranatum* extracts. The antimicrobial effectiveness of the plant can be attributed to these phyto-constituents, which may exert their effects through various mechanisms, including disrupting the cell membrane, inhibiting cell wall formation, inactivating microbial adhesins, suppressing enzymes, or impeding nucleic acid synthesis (Cowan, 1999). Furthermore, research has demonstrated that phytochemical substances can disrupt the operations of multidrug-resistant (MDR) microbes by influencing their efflux pumps, resistance plasmids, beta-lactamase enzymes, and bacterial gene transposition (Mahindra and Kateryna, 2013). Specifically, the methanolic extract exhibited the highest activity against all bacterial strains tested, displaying a zone of inhibition measuring 16 mm against *Pseudomonas aeruginosa*, 19 mm against *Escherichia coli*, 16 mm against *Staphylococcus aureus*, 16 mm against *Bacillus subtilis*, and 14 mm against *Aeromonas hydrophila* at a concentration of 100 mg/ml. Similarly, the acetone extract also demonstrated significant activity, with zones of inhibition measuring 15 mm against *Escherichia coli* at the same concentration of 100 mg/ml. In contrast, the negative control (10% DMSO) demonstrated no activity against any of the bacterial strains under examination. These findings were compared to those of the positive control (Gentamicin), which displayed inhibition zones measuring 25 mm for *Bacillus subtilis* and *Pseudomonas aeruginosa,* 26 mm for *Staphylococcus aureus*, and 20 mm for *Escherichia coli.* Furthermore, the methanolic extract exhibited significant activity against all tested bacterial strains, demonstrating inhibition zones measuring 15 mm against *Pseudomonas aeruginosa*, 17 mm against *Escherichia coli*, 18 mm against *Staphylococcus aureus*, 18 mm against *Bacillus subtilis*, and 14 mm against *Aeromonas hydrophila* at a concentration of 100 mg/ml. The acetone extract also displayed considerable activity, with zones of inhibition measuring 15 mm against *Escherichia coli* at the same concentration of 100 mg/ml. The negative control (10% DMSO) had no effect on any of the bacterial strains tested. In the case of *Myriophyllum spicatum*, the results were compared to the positive control (Gentamycin), which demonstrated a zone of inhibition measuring 25 mm against *Bacillus subtilis* and *Pseudomonas aeruginosa*, 26 mm against *Staphylococcus aureus*, 20 mm against *Escherichia coli*, and 25 mm against *Aeromonashydrophila*. Our findings regarding the antibacterial activity of various plant extracts are consistent with prior studies where the methanolic extract exhibited the highest activity (Kavishankar *et al.,* 2011; Swamy *et al.,* 2012; Wani *et al.,* 2013). The choice of solvent significantly affects the antimicrobial activity (AA) of an extract. This variability is likely due to differences in the solubility of various phytochemicals in distinct solvents, influenced by their relative polarities and solubilities, as reported in several studies (Al-Zubaydi *et al.,* 2009; Boklari, 2009; Bakht *et al.,* 2011). While water was traditionally employed for extraction by traditional healers, investigation indicates that the extracting solvent plays a crucial role when analyzing the pharmaceutical properties of a medicinal plant (Parekh *et al.,* 2005). Concerning microbial susceptibility, Escherichia coli and Staphylococcus aureus were the most susceptible bacterial strains. Similar findings were obtained by (Antara and Amla, 2012) in their study on the antibacterial activity of different solvent extracts of *Meliaazedarach*. The minimum inhibitory concentration of the methanolic extract against *Staphylococcus aureus* in *Myriophyllum spicatum* plants was determined to be 50 mg/ml. However, the MIC for the methanolic extract was detectable only at a concentration of 30 mg/ml, remaining undetected in the rest of the test microbes.

## CONCLUSION

The surge in drug resistance among aquaculture pathogens and concerning effects of specific antibacterials underscore the need for novel antimicrobial solutions. Research into the antimicrobial properties of herbs against resistant bacterial strains has regained prominence. Plant leaves, rich in antimicrobial compounds like tannins and flavonoids, offer a promising avenue. Our study specifically extracted *Myriophyllum spicatum* using various solvents and thoroughly analyzed its phytochemical composition. The methanolic extract demonstrated the highest antibacterial efficacy, highlighting the crucial role of solvent selection. There's a growing interest in natural hydrophytic plant extracts as antimicrobials, aiming to combat foodborne diseases and reduce dependence on synthetic drugs. This study underscores *Myriophyllum spicatum* potential as a natural remedy for infectious diseases in aquaculture, exhibiting effectiveness against diverse bacteria and

aquaculture-related pathogens.

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