ISSN No:-2456-2165

Antimicrobial Property of Over-the-Counter Essential Oils and Ayurvedic Powders against *Aeromonas hydrophila* Isolated from *Labeo rohito*

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Abstract:- This study aimed to investigate the antimicrobial activity of Syzygium aromaticum, Lavandula augustifolia, Zingiber officinale and Allium sativum essential oils and also powders of Emblica officinalis and Triphala (Emblica officinalis, Terminalia bellerica and Terminalia chebula) against Aeromonas spp isolates from Labeo rohita commonly known as Rohu. Essential oils were obtained from Khadi India manufacturers and powders from Khadi Natural manufacturers. The antimicrobial activity of the oils was assessed using the disc diffusion assay and the minimum inhibitory concentration was calculated using the simple broth dilution method. Syzygium aromaticum, Lavandula augustifolia, Zingiber officinale, Allium sativum, Emblica officinalis powder, and Triphala powder had MIC values of 500, 500, 350, 25 µg/ml, 5, and 10 mg/ml, respectively. All substances had antibacterial property against Aeromonas hydrophila. Syzygium aromaticum was found to be most susceptible and Triphala powder was least susceptible according to the Disc diffusion method. These products can be used as complementary therapeutic and prophylactic agents against this pathogen, with antimicrobial or immunostimulant properties. However, further research is needed to determine the safety and efficacy of these substances in vivo and their interactions with this bacterium.

Keywords:- *Phytotherapy; Disc Diffusion Method; Aeromonas Hydrophila; Motile Aeromonas Septicemia (MAS); Clove Oil, Ayurvedic Powder.*

I. INTRODUCTION

Aeromonas hydrophila is a motile, Gram-negative, coccobacilli rod that ranges in size from one to three meters, does not produce spores, and have rounded ends. Sludge, rivers, algae, seagrasses, polluted drinking water, and foodstuffs are just a few of the aquatic and ecological habitats where Aeromonas species has been found [1,2]. Gastroenteritis, septicemia, and necrotizing fasciitis are the three most common conditions caused by the freshwater, facultatively anaerobic, chemo-organoheterotrophic bacteria *Aeromonas hydrophila* in fish, amphibians, reptiles, birds, and mammals. [[3],[4],[5],[6]]. An infection with *A. hydrophila* is a zoonotic illness, meaning that it may spread from animals to people and vice versa [7]. These infections can be brought on by stressful situations such as a shortage of space low dissolved oxygen, greater organic content, trauma to the body, climate fluctuations, and manufacturing industries pollution [8,9].

A. hydrophila can be characterized as a primary or secondary pathogen [10,11]. A pathogen is considered a primary pathogen when it attacks stressed fish and does so on its own. It is often discovered as a secondary invader [12]. Secondary pathogens depend upon primary infection for transmission since they have a restricted capacity to attack. Usually regarded as a secondary pathogen, *A. hydrophila* infects fish that have already been subjected to another illness [13]. *A. hydrophila* can also infect fish when they are stressed or in combination with other diseases [14]. It is regarded as a reliable indicator of a contaminated or stressed aquatic environment [15]. The phrase ”opportunistic pathogen” denotes the fact that the bacterium always can spread illness [13].

In the field of medicine, phytotherapy, conventional techniques are prioritized above advanced therapies. It heavily relies on understanding and using medicinal herbalism. Even though the aquaculture sector has only just started employing phytotherapy, it is progressively coming to be seen as a viable alternative to synthetic medications [16]. The word for this eco-friendly and sustainable application is phytotherapy, sometimes known as herbalism. The use of medicinal herbs in aquaculture has drawn considerable interest from all around the world and is currently the subject of in-depth scientific research.[17,18]. For thousands of years, medicinal plants have been used in traditional medicine all across the world.

These plants have a wide variety of qualities that are appetite-stimulating, growth-promoting, antibacterial, immunostimulant, anti-inflammatory, antistress, and anti-cancer. They do not appear to have any negative side effects, in contrast to therapies like chemotherapy. Additionally, they play an integral part in the creation of rural, economically viable neighborhoods and are easily accessible, cost-effective, and biocompatible. [19].

This study aimed to explore the unexplored common drugs of the herbal formulations of Ayurvedic and Essential oils and to examine the effect of these formulations against the growth of the bacterium.

ISSN No:-2456-2165

II. MATERIAL AND METHODS

A. hydrophila strain ATCC-49140 were used throughout this study. ATCC-49140 was originally obtained from the from the gills of Rohu fish (*Labeo rohito*) with clinical symptoms of Motile Aeromonad Septicemia (MAS) which is characterized by Systemic infections and septicemia, or the presence of germs in the bloodstream. Fishes can acquire bacteria through wounds, their gills, or their digestive systems [20]. This strain was confirmed by positive catalase and oxidase test.

A. Preparation of samples

Essential oils (Clove oil, Lavender oil, Ginger oil and Garlic oil) were obtained from were obtained from Khadi Natural manufacturers. Suitable dilutions of the oils were prepared in 99.7 percent filter-sterilized ethanol. Ayurvedic powders (Amla powder and Triphala powder) were obtained from Khadi Natural manufacturers. Suitable dilutions of the Powders were prepared in Distilled water.

B. Bacterial growth inhibition test of herbal formulations (Essential oils and Powders) by the disk diffusion method and Minimum inhibitory concentration

The tested strain was plated on nutritional medium and cultured for 24 hours at 25°C. Following that, distilled water was added to the microorganism suspensions, and the turbidity was adjusted to meet the 0.5 McFarland criterion. 400 µl of standardized bacterial inoculum (10^8 CFU ml-1) were added to nutrient agar plates, which were then dispersed using sterile swabs. Each of the culture plates was covered with sterile filter paper discs that had been impregnated with formulations 15 minutes after the bacteria solution had been deposited. The Kirby-Bauer disc diffusion susceptibility test methodology was used to conduct the antimicrobial susceptibility testing on Nutrient agar[21]. In each experiment, a disc immersed with sterile ethanol served as the negative control. The diameters of the control and standard susceptibility discs were compared to those of the millimeter-sized inhibitory zones. The existence of inhibitory zones around the well-indicated activity. Three repetitions of each test were performed, and then the average antimicrobial activity values were computed. To determine whether bacteria were susceptible to or resistant to the tested phytochemicals, the zone diameter criteria listed below were used: 15 mm is considered Susceptible (S), 11–14 mm is Intermediate (I), and 10 mm is Resistant (R) [22].

Using the broth dilution technique, microbial cultures were made by suspending two to three isolated colonies from a grown-up petri dish in 50 ml of LB broth. After 24 hours, the suspension was diluted using the 0.5 Mc Farland standard to obtain the final inoculum population ($5x10^5$ CFU/ml). Each test sample was then serially diluted in test tubes using LB broth and known stock solutions, with each tube receiving an identical amount of bacterial inoculum ,i.e., 1 ml and a total of 10 ml of solution in the test tube, made up of 9 ml of serially diluted samples of ayurvedic powders and essential oils. The MIC value was established as the lowest chemical concentration that stopped apparent bacterial growth in the medium, as shown by the lack of turbidity.

III. RESULTS AND DISCUSSION

Table 1 displays the information on the formulations' antibacterial potential. Table 1 compares the types of susceptibility for the disc diffusion technique, including susceptible, moderate, and resistant. The results indicated that the *A. hydrophila* (400 µl of standardized inoculum) revealed high susceptibility to essential oils of *Syzygium aromaticum, Zingiber officinale, and Lavandula augustifolia*. Intermediate susceptibility to *Emblica officinalis* solution herbal formulations, *Allium sativum*, Triphala (*Emblica officinalis, Terminalia bellerica, and Terminalia chebula*) solution. There was no resistance found against any of the herbal formulations.

For MIC, *Emblica officinalis* (Amla) and Triphala powders were found to have significant antibacterial properties against *Aeromonas hydrophila* at concentrations of 5 mg/ml and 10 mg/ml, respectively as presented in Table 3. These results suggest that these natural remedies could be used as an alternative to traditional antibiotics, which can adversely affect human health and contribute to the development of antibiotic- resistant bacteria. Further research is needed to evaluate their efficacy in treating microbial infections in vivo. Similarly, *Syzygium aromaticum* (clove) oil was found to be a potential natural substitute for conventional antibiotics in the treatment of *Aeromonas hydrophila* infections, with a MIC value of 500 µg/ml. *Lavandula augustifolia* (Lavender) oil was also found to be effective against this bacterium, with a MIC value of 500µg/ml. *Zingiber officinale* (Ginger) oil showed promising results, with a MIC value of 350 µg/ml against *Aeromonas hydrophila. Allium sativum* (Garlic) oil was also found to have potential antibacterial properties against *Aeromonas hydrophila*, with a MIC value of 25 µg/ml as presented in Table 2.

ISSN No:-2456-2165

A. Tables

TABLE I. SUSCEPTIBILITY OR RESISTANCE OF *A. HYDROPHILA* AGAINST ESSENTIAL OILS AND AYURVEDIC POWDERS OBTAINED FROM MARKET

SUSCEPTIBILITY OR RESISTANCE OF *A. HYDROPHILA*			
OILS AND POWDERS	*SUSCEPTIBLE (S) ≥15 MM*	*INTERMEDIATE (I) = 11-14 MM*	*RESISTANT (R) ≤ 10 MM*
SYZYGIUM AROMATICUM OIL	+		
ZINGIBER OFFICINALE OIL	+		
LAVANDULA AUGUSTIFOLIA OIL	+		
ALLIUM SATIVUM OIL		+	
EMBLICA OFFICINALIS POWDER		+	
TRIPHALA (*EMBLICA OFFICINALIS*, *TERMINALIA BELLERICA* AND *TERMINALIA CHEBULA*) POWDER		+	

TABLE II. EFFECT OF DIFFERENT CONCENTRATIONS OF ESSENTIAL OILS AGAINST *A. HYDROPHILA*

A. HYDROPHILA INITIAL POPULATION (10^8 CFU ML^{-1})				
Concentration of essential OILS-IN (µG/ML)	CLOVE OIL	LAVENDER OIL	GARLIC OIL	GINGER OIL
10	ND	ND	+	ND
25	ND	ND	-	ND
50	ND	ND	-	+
100	+	+	-	ND
200	ND	ND	ND	+
250	+	+	ND	ND
350	ND	ND	ND	-
500	-	-	ND	-
750	-	-	ND	ND

TABLE III. EFFECT OF DIFFERENT CONCENTRATIONS OF AYURVEDIC POWDERS AGAINST *A. HYDROPHILA*

A. HYDROPHILA INITIAL POPULATION (10^8 CFU ML^{-1})		
Concentration of Ayurvedic POWDERS IN (MG/ML)	*AMLA*	*TRIPHALA*
1	+	ND
5	-	+
10	-	-
15	-	-
20	ND	-
25	ND	ND

NOTE:
+ : Visible Growth
- : Growth inhibited
ND : Not done

IV. CONCLUSION

The overuse of antibiotics and other synthetic drugs causes the emergence of antibiotic- resistant strains and the accumulation of drug residues in fish tissues that are afflicted with infectious diseases like Motile Aeromonas Septicemia (MAS) and in the water, which may be hazardous for the environment and harmful to consumers. Medicinal plant-derived products appear to be a practical tool for enhancing growth, survival, health status, innate and immunological responses, as well as disease caused by *A. hydrophila* species, in addition to vaccination and conventional treatments. This is because powerful bioactive chemicals are readily available. These are also easily accessible, quite affordable, and nontoxic[23].

The most effective compounds/metabolites that could be used to control the infections caused by A. hydrophila in both humans and all aquaculture species require further study in order to determine the ideal administration doses and timings as well as to separate, characterize, and quantify the bioactive compounds found in plants and phytoextracts[23].

ACKNOWLEDGMENT

The authors are grateful to the management of the Amity Institute of Biotechnology, Amity University to provide the lab facility and Dr. Shail Pragya Singh for her guidance during the tenure of this research work.

ISSN No:-2456-2165

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