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The Role of Allium sativum Extract in Treating *Aeromonas* Infection of *Labeo rohita*

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ABSTRACT

The *Aeromonas* species of gram negative bacteria is a motile, pathogenic species which is generally seen in the various bacterial diseased fishes that are known to host various fresh and brackish water fish species that are generally seen in abundance globally and are generally found in cold to temperate zone regions and seen to be cultured in the warm water fish. The content of interest in this study is to analyze the inhibitory properties of the phytochemical extract of the *Allium sativum* against the *Aeromonas* species of diseased host fish *Labeo rohita*. Extracts of above that are sensitive to *Aeromonas* are taken and minimum inhibitory concentrations (MIC) of the extracts were added and analyzed by various qualitative methods. The results and data can then be analyzed statistically using ANOVA and the phytochemical screening shall be done using the standard procedures of estimation. Extracts of aforementioned phytochemicals are seen to possess the potential as therapy against *Aeromonas* which causes Aeromoniasis of Rohu that are having an opportunistic and global status and whose cure and inhibitory effect by the plants natural defense system over Aeromonas shall show a great advantage for various aqua culturists in better progress and production in its part as Rohu being a freshwater fish is a food for many. In this assessment, the vital organic functions of *Allium sativum* L. are briefly highlighted with supportive working mechanisms. Further, the mechanism is then discussed how it is seen to be applicable on the treatment of Aeromonas causal diseases of *Labeo rohita*.

KEY WORDS: AEROMONAS, ROHU, PHYTOCHEMICALS, AQUA CULTURISTS, OMEGA-3-FATTY ACIDS.

INTRODUCTION

The development of the population has led to rapid growth that has led to competition in every sphere of life i.e.; more land, more resources of everything, more food etc. So, the fittest is always considered to thrive in this competitive world and thus, for the present scenario there is an increasing demand for the basic materials like the food and the food products and where in one sphere is the aquatic ecosystem is coming into great demand thus, the seafood is acquiring its popularity too. Freshwater is seen to add up a larger amount of its production day by day in the diet conscious and developing scenario of the world food production both in variety as well as biomass. The freshwater or the anadromous fish is seen to be a better option as compared to the meat as it has a lesser lipid content and more unsaturated fat and rich source of omega -3-fatty acids and aids better digestion due to the presence of various digestible protein in it. The major producers of aquaculture species are identified as Asia, Europe and Africa (Mazumdar et al., 2015).

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Rohu (*Labeo rohita*) a major Indian carp is one of the most commercially significant species in this group of freshwater species and also a very popular cuisine ingredient in the Indian communities (Mazumdar et al., 2015). But the various studies have shown that the Rohu commercialization in the aquaculture sector is having a persistent threat because of various pathogens like the *Aeromonas* species. The bacterial *Aeromonas* species which are the rod shaped bacterial pathogen that have been generally identified as a potential pathogen in causing infections not only in the fishes but also in various amphibians , reptiles but also affect the humans as well as the former organisms act as a carrier of that infection. The infectious pathogen.

Aeromonas is generally isolated from the surface waters, estuarine water, gills , eggs, freshwater, ventral muscles and stomach of fresh water prawns(Rahim, & Aziz, 1994), various consumable products ,in the diseased or healthy fish,excretory products of hominids and animals, thus they have concluded by various studies to be omnipresent in the aquatic ecosystems(Dar et al., 2016).Further they are also cultured in an environment where warm water fish thrive so , they are generally seen to be everywhere as mentioned before too(Chopra et al., 1993) (Nordmann & Poirel , 2002). Further the *Aeromonas* is of no commercial advantage as various studies indicate it as a "Developing foodborne pathogen" as at ambient temperature and ubiquitous nature gives it a great boost in it developing as an active spoiler of fish and meat. The conditions if are favorable too also as they enhance the chances of better disease development by these species in the fish due to the stress situations like overpopulation(because of the method like polyculture of fish) , rough handling , cross contamination , meagre water quality thus leading it to cause major epidemic outbreaks and causes various biological hazards.

Over several years it has been seen through global studies that the seafood is an important foundation of various foodborne outbreaks so, if this foundation is tackled smartly then it won't be able to spoil the environment or cause any detrimental changes in the aquatic ecosystem. According to a study it has been reported that the European Union (EU), in 2015 had many foodborne outbreaks of which 10% has been seen to be linked to the seafood. Moreover the disease is not coming into light due to the various unreported cases (Auth, 2016). Today upto this present time also the awareness on the growth and pathogenic prospective properties of the *Aeromonas* species in the seafood and seafood products that are a major food to be ingested and projected for raw consumption is very limited. Insufficient studies are concentrated w.r.t to the presence of this pathogen in lightly processed Ready-To-Eat (RTE) seafood products such as sushi (Atanassova et al., 2008) (Pinto et al., 2012) (Hoel et al., 2015), cold smoked salmon (Hudson & Mott, 1993) and molluscan shellfish (Silva et al., 2018).

Thus, the dangerous pathogen can be controlled using various plant resources one of which to be studied here is the *Allium sativum* ordinarily named as the garlic i.e. is seen to have a greater application on the fish culture and helps in stimulating the defence system activity as well in a progressive way and it is one such traditional power packed herb that has the antimicrobial activity (Liza et.al, 2018). The various phytochemicals seen in the extraction are the flavonoids, phenolic, saponin, alkaloids and tannins that are seen to show better results in antimicrobial activity through various studies and will help to inhibit the growth of *Aeromonas* bacterial growth in the Rohu fish (Lekshmi et al., 2015). As Rohu is a majorly available fish in Northern as well as Central India and as well its meat is mostly consumed as it is plentifully filled with the vitamins like A(a group of fat-soluble retinoids),B and C(ascorbic acid) and omega -3-fatty acids whose better management against this disease shall give better health benefits to consumer too.

This study here aims to summarize the status of *Aeromonas* as a probable foodborne pathogen, and to deliberate the importance of *Allium sativum* in the enhancement of defence system of Rohu and thus, inhibits the bacterial growth and thus help in the better commercial benefits in the field of aquaculture by use of completely natural, traditional herb with least side effects and provide benefit as a whole thus providing welfare of human, and protect the environment and aqua species as well thus showing largely, the garlic as a tremendous regularly ingestion food source as it's a power pack ingredient possessing multidiverse bioactive sulfuric compounds and that has encouraging claims in the advancement of this as a great functional foods or nutraceuticals for the better management of the fish diseases like Aeromoniaisis, Epizootic ulcerative syndrome, fatal septicaemia, ascitis , ulcerations etc., so that this natural ingredient being a traditional herb can be given inputs as a nutritional ingredient to save the aquatic beings and the organisms depending on that too.

2. Rohu (*Labeo rohita*)

2.1 Scientific name: *Labeo rohita* (Hamilton, 1822)

2.2 Taxonomic Classification

Phylum	Vertebrata
Subphylum	Craniata
Superclass	Gnathostoma
Series	Pisces
Class	Teleostomi
Subclass	Actinopterygii
Order	Cypriniformes
Division	Cyprini
Suborder	Cyprinoidei
Family	Cyprinidae
Subfamily	Cyprinini
Genus	*Labeo*
Species	*rohita*

2.3 Morphological characteristics

2.3.1 Scales

- Moderate in size.

- Found along the lateral line with the presence of around 40 to 42 scales.
- Scales are seen to be arranged in a transverse manner from dorsal fin origin to the base of ventral.
- Around the predorsal scales 14-17 scales are found and along the caudal fin there are 20 scales

2.3.2 Barbels

- Concealed in the lateral grooves are a pair of short, thin maxillary barbles.

2.3.3 Measurements

- The fish has a head of about 4.5 to 5, depth of 4 to 4.7 in length.
- Anterior half of head has the eye located in it
- Obtuse snout devoid of labial folds with an inferior mouth and distant inner fold to each lip.

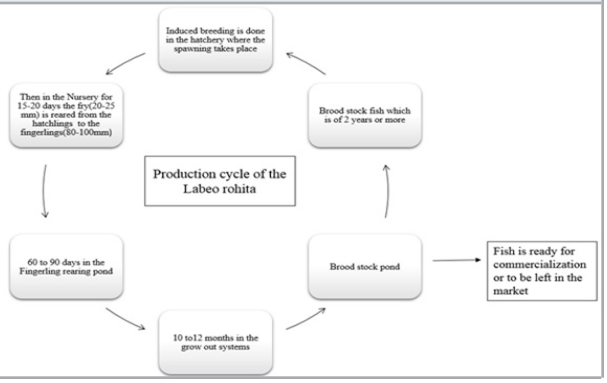
2.3.4 Colouration

- Back is observed to be bluish with sides and beneath having a silvery colour .
- Breeding season brings in a rod shaped marking on each scale with black and greyish fins.
- Body colour is changeable according to its habitat i.e. greenish black back observed in the fishes living among the weeds
- Reddish eyes are studied.

2.3.5 The production and harvesting of Rohu fish

- When quite small they are generally cultured in the aqueous environment in pond and tanks.
- A type of technique called repeated netting is seen at the end of the fish culture period.
- Almost all the seed required for the culturing of fish is done through induced breeding of Rohu.
- Hypophysation is a common practice done here since 1957, several synthetic commercial formulations.
- For seed production a common hatchery that's used here is a circular one constituting of the spawning or the breeding tank, hatching or the incubation reservoir and further stored water and supply system is done before hand.
- The weight ratio of the female is to male ratio is normally kept at 1:1 and 1:2 by number.
- For next 15 to 20 days a nursery stage is done for raising fry, followed by a two to three months phase for fingerling development.
- Then the fingerlings undergo rearing phase where the 6mm three day old hatchlings, , are reared to become a fry of 20-25 mm in small earthen nursery ponds of 0.02-0.1 ha.
- The nursery-raised fry of 20-25 mm are further reared for two-three months to 80-100 mm (6-10 g) fingerlings in earthen ponds of 0.05-0.2 ha.
- Around 60 to 70 percent fingerlings are seen to survive and then the Rohu is grown in such a way and then followed by harvesting.
- Several stocking in addition to harvesting procedures are practised, the size is seen and the harvesting of larger sizes (300 - 500 g) is commonly begun after six-seven months of culture, and the smaller ones are sent back to the pond for better progressive size attatinment.

Figure 1: Production Cycle of Rohu



2.3.6 Handling and processing: Among the major Indian cultivated carps Rohu is seen to be a significant species. Even these species are found to be large commercialised and fresh water species which are further cleaned by water thoroughly, and packed along with crumpled ice pieces in 1:1 ratio inside rectangular plastic crates (frequently of 60 cm x 40 cm x 23 cm size). Packed fish with ice are sent to longer distances in proper shielded vans throughout India around 3000 km by land transport of these species. Some non-existent and value-added fortification method are still non-existent in such high marketing countries of this species of Rohu.

2.3.7 Production costs: Rohu being a low valued species is always tried to give minimum input costs and a minimum of the supplementary feed, seed , fertilizers are tried to be utilised with maximum overturn.

3. The biology of Aeromonas species: The *Aeromonas* is generic name which comes under one of the five genera of the family of Aeromonadaceae., order Aeromondales ,class Gammaproteobacteria, Phylum Proteobacteria and thus belongs to the Kingdom Bacteria which was once considered as part of the family Vibrionaceae, which has two genera, namely, Vibrio and *Aeromonas* (Inglis et al., 1993). It is found to be gram-negative autochthonous aquatic bacilli, which are oxidase-catalase-positive and facultative anaerobe. Generally the members of this genus are categorised into 2 major groups ,one of which is immotile, fish-infecting, psychrophilic immotile strains that comprises of *Aeromonas* salmonicida.

The extra larger group comprises of larger, motile, mesophilic *Aeromonas* species, and generally infect the humans (Martin et al., 2005 , Martino et al., 2014 ,Pang et al., 2015).Reclassification of this species is going on as described above. The 2005 type of the Bergey's Manual of Systematic Bacteriology, described that there were around 14 species under this genus and formerly known as the Enteric group 501, then later on categorized as *Aeromonas* diversa (Martin et al., 2005 , Minana et al., 2010) Old classification was differentiated based on the 16S rRNA of the and DNA-hybridization groups (Coloston et al., 2018). So far, *Aeromonas* are having 36 species described since 1943 - out of which 19 are

detrimental and human pathogens (Hoel et al., 2019). The various species are listed below in the underlying table1.

Incidence of Aeromonas diseases in Rohu: In these recent years of detailed fish study the aquaculture zone where the various fishes, shrimps, humans, etc. are being affected due to the various pathogens of which a dangerous non-commercial pathogen of the *Aeromonas* spp. is causing a persistent hazard due to this fish pathogen. According to a study done by Gowhar et al., 2016, various biochemical studies are a crucial part in the management of various fish pathogens like of the *Aeromonas* spp. As we know the pathogens which are disease causal agents are generally saprophytic in nature and they evolve into pathogenic species only if there is certain negligent practices, higher pollution levels, unbalanced physiological conditions, nutritional deficiency or other stress enhancers like the non-optimum water quality, congestion and various anthropogenic activities which are great opportunities to trigger the *Aeromonas* infections in fish in the field of aquaculture (Mishra et al., 2017).

Figure 2: Phytochemicals in Garlic (Bautista et al., 2005)

Name of the species	References
Aeromonas allosaccharophila *Aeromonas aquatica* *Aeromonas finlandiensis*	Martinez et.al,1992 Beaz et.al,2015
Aeromonas aquatilis, *Aeromonas crassostreae,* *Aeromonas enterica, Aeromonas*	Figueras et.al,2017
Aeromonas australiensis *Aeromonas bestiarum*	Roman et.al,2013 Ali et.al,1996
Aeromonas bivalvium	Galbis et.al,2007
Aeromonas cavernicola	Martinez-Murcia et.al,2013
A. caviae , Aeromonas eucrenophila	Schubert et.al,1988
A. dhakensis	Beaz-Hidalgo et.al, Huys et.al, 2002 Martinez-Murcia et.al, 2008,2013
Aeromonas diversa	Miñana-Galbis et.al,2010
Aeromonas encheleia	Esteve et.al,1995
Aeromonas fluvialis	Alperi et.al,2010
A. hydrophila	Stanier et.al,1943
Aeromonas jandaei, Aeromonas trota	Carnahan et.al,1991
Aeromonas media	Allen et.al,1983
Aeromonas molluscorum	Minana Galbis et.al,2004
Aeromonas lacus, Aeromonas lusitana	Martínez-Murcia et.al,2016
Aeromonas piscicola	Beaz-Hidalgo et.al,2009
Aeromonas popoffii	Huys et.al,1997
Aeromonas rivipollensis	Marti et.al,2015
Aeromonas rivuli	Griffin et.al,1953
A. salmonicida	Griffin et.al,1953
Aeromonas sanarellii	Alperi et.al,2008
Aeromonas schubertii	Hickman-Brenner et.al,1988
Aeromonas simiae	Harf-Monteil et.al,2004
Aeromonas sobria	Popoff et.al,1981
Aeromonas taiwanensis, *Aeromonas tecta*	Demarta et.al,2008
A.veronii	Hickman-Brenner et.al,1987

The various parameter based studies done by Gowhar et al., (2016) in the pond environment further explored during the present study was thus showed that the field of aquaculture is prone to a variety of diseases due to inapt farm management systems, and further the various fish species mostly the study they did was *Labeo rohita* showed greater susceptibility of fish to get attacked by various pathogenic infections is enhanced (Sayed, 2006) and variety of movable *Aeromonas* Spp. has been thus detected in the aquatic fish and its habitat (Kaper et al.,

1981; Carlos et al., 1986; Hatha et al.,2005). Various human activities leads to the spread of thus pathogen from one organism to other similarly such kind of scattering when seen in the pathogenic organism like A. sobria by excreta can completely disturb the entire pond ecosystem and be carried through food chain to various organisms and thus reach the humans thus leading to a mechanism of plasmid interchange between the bacterial species that freely then keeps on spreading and facilitating its strains elsewhere and can thus result in a higher occurrence of numerous antibiotic resilient strains and the development of this fish disease in the fish (Chang & Bolton , 1987).

Medicated supplements to treat this cycle further helps the pathogen of *Aeromonas* to spread and further lead to an outbreak in the waterbody by the further production of various virulent and resistant strain types(Gowhar et. al.,2016) (Redmayne,1989). *Aeromonas* species leads to septicaemia with extensive skin lesions, dermal oedema , musculature disintegration is seen and worsening of the internal organs like liver, spleen and muscles. The visible symptoms of the diseased fish include lethargic movement accompanied with pale red gills, blackening at the scraped areas. Tissue sections further revealed focal haemorrhage and various necrotic changes in the haematopoietic tissue of the liver, glomeruli and renal tubules in kidney, hyperplasia of gill lamellae, swelled intestinal mucosal epithelium and mild deteriorating changes of myocardium which are generally observed by the fish affected by the *A. hydrophila* type (Manoj et al., 2010). According to the studies done by Gowhar et al., 2016 the results they obtained from the Vitek database directed the probabilities of various species of *Aeromonas* as of identification of then they have concluded to have found about 95 to 99 percent of A. veronii and a percentage of 69 to 83 percent of the species of *A. hydrophila*, *A. caviae* and *A. sorbia* (Cai et al.,2012) and about 93 percent of the A. sobria (Dar et al., 2016).

4.1 Isolation and Identification of Aeromonas: From a study done by Fricker and Tompset out of 563 fish and meat samples were obtained from retail outlets and investigated and concluded the isolation of mesophilic *Aeromonas* species from around 287 samples .The symptoms of *Aeromonas* disease affected fish species were observed by them and were further isolated from gills, eggs, stomach and ventral muscles of fresh water prawns available in Bangladesh (Zeaur & Aziz, 1994). A study on food mock-ups showed a 10.47% percent positive results for aeromonads. Out of 99 fish samples 22 (22.22%) were found positive, in which *A. hydrophila* (66.6%), A. sobria (27.27%), A. caviae (9.09%) were found positive (Leitao et al., 1991).

Further experimental studies done by Gowhar et al., showed that the characterisation of being *Aeromonas* species is primarily done by seeing the various symptoms that this *Aeromonas* bacterium shows on the *Labeo rohita* then they isolated the fish pathogenic bacteria by using a culture dependent approach by using a spread plating technique .Later on they swabbed the fish surface for bacteria segregation, and then the inoculums were spread over the agar rich nutrient medium (Dar et al.,2016, Jingram 2007 ,Tilak 1987, Noor et al.,2014 ,Austin & Austin,2012) and kept it at 25°C-30°C incubation for a duration of 2-3 days (Spanggard et al.,2000; Harbi & Uddin 2004 ,2005). Further they isolated and purified the stocks and further the morphological and biochemical characteristics analysis done by them showed the *Aeromonas* species on the nutrient agar plate. In this way they are detected over the fish species as they did in the case of *Labeo rohita*.

4.2 Treatment of Aeromonas diseases in Labeo rohita: Aeromoniasis, a disease cause by *Aeromonas* species is a gram negative, facultative anaerobe or food borne pathogen among the aquatic species that has caused great menace and havoc among the aquatic ecosystem. No epidemiological evidences are actually in availability so its very difficult to treat or completely eliminate the once culture facilities and/or aquatic species that are infected (Chauhan, 2014; Angahar, 2016; El-deen et al., 2018). Aeromoniasis or motile aeromonas septicaemia disease, tail rot and fin rot, eye and ulcer diseases in fish hatchery can be effectively controlled by removal of infested eggs from the troughs or incubation gutter. Prevention is the better method of controlling this disease.

Therefore, the diseases caused by the *Aeromonas* can be best prevented by good aquaculture management practices such as:

- Ulcers could be prevented by removal or complete terminalisation of the diseased fish, also the ponds should be sterilised with a solution of potassium permanganate at 0.5 parts per million ,and supplemented feed of sulphadiazine at 100mg/kg or terramycin 75-80 mg/kg could be given for a period of 10-12 days.
- Dropsy could be prevented by sterilizing the pond with the potassium permanganate solution of quantity 1 parts per million and potassium permanganate bath treatment of 5 ppm for a time interval if 2 minutes.
- Epizootic ulcerative syndrome could be prevented by the lime usage of quantity of 200kg/ha and application of 0.1 ppm of CIFA ,India product called CIFAX .
- Proper pond sanitation is the key to prevent such pathogens growth.
- Motile *Aeromonas* Septicaemia can be treated by using medicated ration containing 2 to 4 g of oxytetracycline/Kg of feed per day for 10 days.
- Experimental vaccination for prophylaxis against infection of *A. hydrophila* has against infection of *A. hydrophila* has been examined.
- Fish immunized either intramuscularly or intraperitoneally with vaccine showed protection against challenge. The agglutinating antibody titter increased in the serum of immunized fish but no commercial vaccine has been developed.

4.2.1 The application of antibiotic and chemotherapy in the management of Aeromonas: As the world is

progressing there is a greater demand for modernised methods where the application of antibiotics and chemotherapy is in demand for its effective property to deal with various types of fish disease's treatment and its prophylaxis .The valuable inputs of antibiotics and chemotherapy is due to its faster results that stands as a greater advantage of this type. But the major obstruction is its environmental threats and the development of antibiotic resistance where the specific *Aeromonas* species has learnt the skills to defeat the drugs designed against them. As the controlling of the *Aeromonas* species in aquaculture observed that *A. hydrophila* isolates from contaminated aquatic species were not sensitive to chloramphenicol. Further changes in the outer cell membrane and extrinsic proteins is observed in the *Aeromonas* salmonicida due to the opposite reaction of quinolones and tetracycline on it and with numerous attempts of resistance of it towards the pathogen (Barnes et al., 1990; Wood et al., 1986). The extensive use of antibiotics against bacterial pathogen thus, helps in the development of resistance by helping them knowing the nature of drugs.

Earlier when multi-resilient *A. hydrophila* were seen to be resistant to antibiotics like penicillin and ampicillin, but sensitive towards chemical agents like aminoglycosides, tetracycline, chloramphenicol, trimethoprim-sulfamethoxazole, quinolones, and second- and third-generation cephalosporin but the frequent usage of these agents leads the pathogen to develop better resistance globally (Igbinosa et al., 2012; Vivekanandhan et al., 2002). In a study done by Parker and Shaw (Parker and Shaw, 2011) many virulent factors like hemolysins, aerolysins, proteases, adhesins, enterotoxins, phospholipase and lipase helped in developing the resistance. As a study done by Stratev and Odeyemi (2016), a table below has described how the *Aeromonas* species in *Labeo rohita* is seen to have developed the resistance against the following antibiotics as described in Table 2.

Table 2. Antibacterial agents against Aeromonas

Anti-bacterial agent against Aeromonas spp.	Resilient strains in % o btained from Labeo rohita
Amoxicillin	52.7
Ampicillin	100
Chloramphenicol	5.5
Nalidixic acid	1.8
Novobiocin	94.5
Oxytetracycline	40
Polymyxin-B	41.8

So, whatever ways as explained the use of antibiotic or chemotherapy or chemical agents like potassium permanganate ,etc uses in spite of its safety may be limited in its applicability due to the high cost of acquiring effective concentration of which is as high as 30,000 mg/L (Hashemi et al., 2012).Many rules and regulations are to be followed with the usage of such agents mostly to be taken care by the aqua culturists and in most cases users often misuse or overuse the required dosage which may cause the target pathogen to develop resistance to the chemical antibiotic applied. Chemicals being in addition, these chemicals are theoretically seen to be hazardous to the users, the fish, the consumers and ultimately the environment and leading to a complete imbalance of the complete ecological balance .Moreover these dangers calls for an urgent steps for the safety of consumers of such fish and our dear environment as the world is on its path of destruction due to various anthropological behaviours.

5. Alternative Treatment of Aeromonas Bacterial Diseases of Labeo rohita by phytochemicals of Garlic (Allium sativum): The plants and the plant products are considered to be the best alternative therapy in the treatment of the various diseases in the aquaculture industry due to the increasing drug filtrate deposition and development of new resistant pathogens in the treated fish due to application of various antibiotics and chemical therapies in the treatment of bacterial diseases and also pose a greater health hazard to the humans and other animals who come in contact with the diseased fish. It is imperious that an alternate treatment for *Aeromonas* is to be industrialized, such that they are an ecologically safe, effective and economically feasible technique of disease management. Many peasant local fish sellers and poor farmers are not able to afford such costly and high paid antibiotics or drugs so, the use of medicinal plants or herbs is moreover, a better option as it will increase the period of production which will ultimately increase the production cost. So, plants being a relatively, non-toxic option, and the fish can be sold and treated at any time and thus, are found to be very safe to be consumed at any time (Ilondu et al., 2009). Other advantages of medicinal plants are plant materials are inexhaustible, harmless, readily available, decomposable and can be treated as food for fishes.

One such anti-phiogistical, seasoning herb is the Garlic (*Allium sativum*) which is the pleasantly available highly pungent smelling natural antibiotic against the bacterial *Aeromonas* spp. in *Labeo rohita*. Garlic (*Allium sativum* L.) is a extensively consumed spice all over the world which is a rich source of various diverse bioactive phytochemicals compounds, such as allicin, alliin, diallyl sulfide, diallyl disulfide, diallyl trisulfide, ajoene, and S-allyl-cysteine. Significant research done on the garlic shows that they are a power pack of bioactive ingredients that are seen to reveal various cure against the most dangerous comorbidities as it acts a great antioxidant, anti-inflammatory agent , anti-bactericidal, anti-fungal, cardiovascular, immune-stimulating , anticancer, Hepatoprotective, gastro protective, anti-diabetic, anti-obese, neuroprotective, and renal protective possessions.

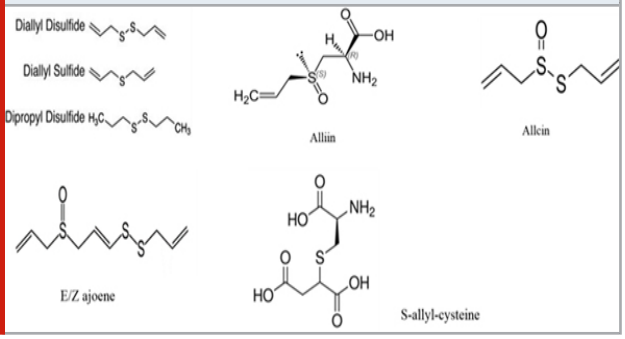
It is powerful ingredient with various organic sulphides, saponin, phenolic complexes, and polysaccharides

(Bose et al., 2014, Diretto et al., 2017, Sahi & Bansal, 2020).It is even a known traditional medicine in China (Jacob & Narendhirakannan, 2019). In comparison to the fresh garlic ,the black garlic type is being greatly focused as they have, higher level of polyphenol and flavonoid content, in addition to restored antioxidant properties(Kimura et al.,2017).

Table 5.1. Systematic classification of *Allium sativum* L. (Garlic)

Kingdom	Plantae
Sub-kingdom	Tracheobionta
Super-division	Spermatophyta
Division	Magnoliophyta
Class	Liliopsida
Subclass	Lilidae
Order	Liliales
Family	Liliaceae
Genus	*Allium*
Species	*sativum*

Figure 2: Phytochemicals in Garlic (Bautista et al., 2005)



5.2. Important bioactive compounds or phytochemicals of Garlic (Allium sativum): Diallyl thiosulfonate (allicin), , diallyl disulfide (DADS),dipropyl sulphide , E/Z-ajoene, S-allyl-cysteine (SAC), diallyl sulphide (DAS) , diallyl trisulfide (DATS)and S-allyl-cysteine sulfoxide (alliin) are the major organosulfur compounds found in garlic (Yoo et al.,2014 , Kodera et al.,2017 , Yoo et al.,2014 , Mansingh et al.,2018).

5.3. Biochemistry of Garlic (Allium sativum) showing the presence of various chemicals: Furthermore, analysis 85 percent fructose, 14 percent glucose, and 1percent galactose were found in garlic. It was known through many research studies that normal raw garlic when given thermal treatment changed to black garlic (Liang et al., 2015). Further thermal processing of black garlic further reduced the sugar levels in it. Also, higher temperature and lower humidity levels enhanced the contents of polyphenols and the total flavonoids in black garlic (Kim et al., 2013). Various studies also suggest that the garlic chemical analysis also suggest that around contains thirty three sulfur compounds, numerous enzymes,seventeen amino acids, and minerals like selenium. The pungent smell and the medicinal effects of garlic is specially due to S-compounds present in it.

One of the most important compounds,is found to be allicin (diallylthiosulfinate or diallyl disulfide) which is obtained only when garlic is crushed ; as an injury to the garlic bulb activates the enzyme alliinase, which metabolizes alliin to allicin. Allicin is later broken down to vinyl thinner which occurs within hours at room temperature and within minutes during cooking. Allicin, which was first chemically isolated in the 1940's, has antimicrobial effects against many viruses, bacteria, fungi and parasites. Garlic oil, aged garlic and steam-distilled garlic doesn't have substantial amounts of aliin or allicin, but instead contain various products of Allicin alteration; not a bit appears to have as much physiologic action as fresh garlic or garlic powder (Londhe et al., 2011).

5. 5. Role of Allium sativum in treating Aeromonas infection of Labeo rohita: Garlic has a wide-ranging variety of anti-bacterial benefits as the garlic is generally seen to have natural antibiotic property and is one of the effective natural immunostimulants ,has antioxidant properties (Rahman,2003) It generally, stimulates the phagocytic cells and proliferates the bactericidal activities and stimulates the natural killer cells, complement, lysozyme, and the antibody responses of fish. The stimulation of these immunity of fish is related with increased protection against infectious disease in fish. As studies made by Lau, 1991 the macrophages accelerate phagocytosis under the influence of garlic. The studies made by Martins et al. when fish diets were supplemented with garlic augmented the RBCs number, hemoglobin level, hematocrit, WBCs number, and thrombocyte number. Studies made Nya suggest that garlic supplementation induced significant changes in serum total protein and globulin in rainbow trout (Nya et al., 2009).

Innate immunity , the serum total protein, albumin, and globulin contents also is seen to increase (Jha et al.,2007). Thus , when increased protection against an immediate challenge with *A. hydrophila*, showing the protective properties of garlic and thus, this property of rainbow trout done can be also used to prevent the *Aeromonas* causal infection in *Labeo rohita*. Studies done by Cavallito and Bailey (1944) showed that the antibacterial properties of crushed garlic could be accredited mainly to allicin component of garlic. Studies done by Diab, Nya and Austin (2009) showed the garlic as a best anti-bacterial agent against *A. hydrophila* in freshwater where they had testified that the usage of garlic-complemented diets for 14 days directed to a marked reduction in death with *A. hydrophila* in fishes. 4 percent mortalities were only seen in groups fed 0.5 and 1percent garlic-mixed feed compared to 88 percent mortality in the control group (Nya & Austin,2009).

Table 5.4. Important biological activities of *Allium sativum* (Shang et. al, 2019)	
Antioxidant Activity	Phenols and saponin; raw garlic>> cooked garlic;fermented garlic like black garlic>> crude garlic; anti-oxidant enzymes like heme oxygenase-1 (HO-1), glutamate-cysteine ligase modifier (GCLM) subunit through the nuclear factor erythrobia-2 related factor 2 (Nrf2)-antioxidant response element (ARE) pathway, which sheltered human endothelial cells against oxidative stress.
Anti-Inflammatory Activity	Inflammatory mediators are inhibited by garlic usasge where the mediators could be nitric oxide, Tumour necrosis factor and (Interleukin) and also has a great potential to treat diseases interrelated to inflammation like the auto-immune disease arthritis as the toxic levels are low in this natural ingredient of garlic.
Antimicrobial Activity	Oil obtained from garlic was seen to be the main antimoneran ingredient that terminates the moneran cell wall structure by inhibiting its metabolic pathway.
Modulating Immune System	Polysaccharides of garlic are seen to promote the immunity as it normalizes the expressions of Interleukins(6,10), tumor necrosis factor-α, and interferon- γ in RAW garlic and also macrophages are secreted a lot.
Cardiovascular Protection	A great agent in the keeping our pumping organ fit is by helping in reduction of blood pressure levels, cholesterol levels, and other heart related problems stay at bay due to garlic consumption in the powdered form.
Anticancer Activity	Various cancers like the colorectal , urinary bladder and intestinal related are avoidable due to the Allium intake as it 1. Metabolic regulator of Cancer-causing Substances 2. Cell Growth and Proliferation are blocked by it 3. Inducing Apoptosis 4. Suppressing Angiogenesis 5. Inhibiting Invasion and Migration
Anti-Diabetic Activity	Repressed pancreatic cell wound, oxidative stress, and pathological changes in streptomycin-induced type 1 diabetic rats, were seen that helped in the management of Diabetes-type2.Thus, Allium components manages our sugar levels too thus, preventing the conditions like insulin resistance.
Anti-Obesity Activity	Anti-obese ingredient garlic is seen to act as cutter of a high-fat diet on the weight of body and breaksdown the fat depository adipose tissue in hyperlipidaemia rats. In addition, the oral administration of (Fermented lactic acid bacteria garlic buds) LAFGE reduced the weight of high-fat diet male C57BL/6J mice and reduced their epididymal, retroperitoneal, and mesenteric adipose tissue mass.

Various studies done by other researchers also suggest same results for this like suggested by (Sahu et al.,2007) that infectious *A. hydrophila* which affected the *Labeo rohita* fingerlings, showed that when the 0.1 and 0.5 percent garlic added groups showed the maximum level of survival (85%) as compared to the controller group (57%) .Study done by Aly and Mohamed (2010) also suggested that *O. niloticus* when fed a 3% garlic-supplemented feed showed a considerably better survival rate (85%) after a challenge with *A. hydrophila* . Zhang (2003) studies measured the inhibitory effects of garlic on two isolates of *A. hydrophila*, AH_1 and AH_1, in vitro and found that the minimum inhibitory concentrations (MICs) were 15.6 and 1.95 mg/ml respectively.

When Chen(1977) studied the 50 mg/diet of garlic on the infected Silurus soldatovi meridionalis ,AH_1 strain was seen to be under control but neither isolate was eliminated completely. Experiments done by Rahman et al. (2009) , evaluated the efficacies of antibiotics and medicinal plants on three common bacterial fish pathogens: *A. hydrophila*, P. fluorescens, and E. tarda found that 8 milligram per mL of garlic when given to the young Thai silver barb (*Barbonymus gonionotus*) showed the finest recovery rate of around (90 percent) during the 10-day trial period. A study done by Dash et al.,2014 suggested that the garlic when compiled with mineral oil, showed a modified adjuvant preparation is well proficient of augmenting defence in the *L. rohita* against *A. hydrophila* infection where the garlic was found to intensify the serum agglutinating antibody titer combined with a greater hemolysin movement of the serum and also increased the survival rate post-challenge thus, suggesting the garlic to be an excellent immunoadjuvant as well as its effect on stimulation of specific and nonspecific immune response stimulation , and seen to be a significant response in lesser dose of garlic might be the contribution of one compound or multiple compounds present in garlic.

CONCLUSION

Garlic a great traditional spice with a great power pack plant with various bioactive compounds and thus, has many beneficial activities being a great renal protector , cardiovascular protector, anti-obese , anti-inflammatory , etc. Therefore, garlic and its phytochemicals are encouraging as functional foods or in the field of nutraceuticals for the therapy of diverse diseases. In the studies to be suggested for future, a greater demand for better evaluation, extraction and separation of biological components of garlic for better performance in biological functions are suggested and to check its intake levels at a safety parameter and much more clinical experiments are to be paid attention to the ill effects/protection of garlic.

Thus, the above experimental set ups done by various renowned researchers showed great quality of work and how the *Aeromonas* can be inhibited but can't be totally eliminated from the infected *Labeo rohita* fish and thus, giving us a better aspect to work on so that the field of aquaculture which is facing such a great pressure due to these pathogens by the simplest , traditional ,healthiest , eco-friendly methods, that they are given more preference and opportunities to work on this garlic isolated phytochemicals to combat this infectious up to some extent at least naturally. However, to prove its complete mechanism and its adjuvant potential, advanced study with continuing trials and on detection of the most active compound of garlic is very essential for saving our aquatic nature and for out practical approach towards the protection of the environment of the harsh anthropogenic activities

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