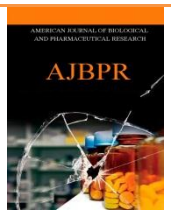
e-ISSN - 2348-2184
Print ISSN - 2348-2176

AMERICAN JOURNAL OF BIOLOGICAL AND PHARMACEUTICAL RESEARCH

Journal homepage: www.mcmed.us/journal/ajbpr



PHYTOCHEMICAL PROFILING AND MOLECULAR DOCKING ANALYSIS OF *Piper nigrum*. L AGAINST ORNAMENTAL FISH DISEASES

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Article Info

Received 29/05/2016
Revised 16/06/2016
Accepted 19/06/2016

Key words: -
Earthworm, *Aeromonas hydrophila*, aerolysin(aerA), *Piper nigrum*, bioactive compounds, Molecular docking.

ABSTRACT

Aeromonas hydrophila is a one of the major fish bacterial pathogens in India, which is more abundant in water. It causes variety of diseases in *Poecilia sphenops* (Black molly) and *Puntius tetrazona* (Tiger barb) fishes. *A. hydrophila* possesses aer A genes which produced aerolysin toxin and that causes haemorrhagic septicaemia, infectious dropsy, tropical ulcerative disease and fin rot leading to heavy mortality in aquaculture farms. Therefore, in the present study aerolysin (aerA) gene act as virulent factor which was docked against bioactive compounds from *P .nigrum*. GC-MS analysis of *P .nigrum* showed 54 Bioactive compounds, among them Piperine showed high energy value -698.77 than other compounds through molecular docking. This research work suggests that Piperine from *P .nigrum* has promising and potent anti-bacterial activities against fish pathogen *Aeromonas hydrophila*.

INTRODUCTION

A.hydrophila is a heterotrophic, Gram-negative, rod-shaped bacterium mainly found in areas with a warm climate. This bacterium can be found in fresh or brackish water. It can survive in aerobic and anaerobic environments, and can digest materials such as gelatin and hemoglobin. It is resistant to most common antibiotics and cold temperatures due to its structure. When it enters the body of its victim, it travels through the bloodstream to the first available organ. It produces Aerolysin, Cytotoxin and Enterotoxin that can cause tissue damage to human and aquatic organisms. *A. hydrophila* is widely considered a major fish pathogen which is associated with diseases mainly found in freshwater fish, because these organisms live in aquatic environment. When infected with *A. hydrophila*, fish develop ulcers, tail rot, fin rot, and hemorrhagic septicemia. Hemorrhagic septicaemia causes lesions that lead to scale shedding, hemorrhages in the gills and anal area, ulcers, exophthalmia, and abdominal swelling. The pathogenicity of *Aeromonas* species was mediated by extracellular protein aerolysin. This toxin has been characterized in *A. hydrophila* which is responsible for severe diseases in fish. *A. hydrophila* infections occur most often during environmental changes, stressors, changes in temperature, in contaminated environments [1].

During those circumstances, Antibiotics such as chloramphenicol, florenicol, tetracycline, sulfonamide, nitrofuran and pyridine carboxylic acids has been used to eliminate the *A. hydrophila* infection [2]. But antibiotics develop resistance to the microorganism [3] Due to these reasons much attention has been paid herbal and traditional medicines in the recent years [4].

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The traditional medicines are being used in a wide range of applications including in the control of bacterial, fungal and viral diseases [5]. Some plants have been screened for their antiviral, antibacterial, antiulcer and anti-inflammatory activities [6-7]. Piperace plants have more potential to cure many microbial disorders and docking is the apt way to screen the plant potentiality to cure diseases

Docking is a process by which two molecules fit together in 3D space. Docking allows virtually screening a variety of compounds and predicting the strongest binders based on various scoring functions [8]. The energy value obtained through docking is used as a criterion for the selection of drugs which involves the identification of lead molecules. The lead molecule is one with maximum interaction showing high negative e-value. Hence the present study focused on Phytochemical profiling and Molecular docking analysis of *Piper nigrum* against ornamental fish diseases

MATERIALS AND METHODS

Gas Chromatography – Mass Spectroscopy (GC-MS) analysis

The powdered sample (20 g) were soaked and dissolved in 75 ml of methanol for 24 h. Then the filtrates were collected by evaporated under liquid nitrogen. The GC-MS analysis was carried out using a Clarus 500 Perkin-Elmer (Auto System XL) Gas Chromatograph equipped and coupled to a mass detector Turbo mass gold – Perking Elmer Turbomas 5.2 spectrometer with an Elite-1 (100% Dimethyl ply siloxane), 300 m x 0.25 mm x 1 x m df capillary column. The instrument was set to an initial temperature of 110°C, and maintained at this temperature for 2 min. At the end of this period, the oven temperature was raised upto 280°C, at the rate of an increase of 5°C/min, and maintained for 9 min. Injection porttemperature was ensured as 250°C and Helium flow rate as 1 ml/min. The ionization voltage was 70 eV. The samples were injected in split mode as 10:1. Mass Spectral scan range was set at 45-450 (mhz). The chemical constituents were identified by GC-MS.

The fragmentation patterns of mass spectra were compared with those stored in the spectrometer database using National Institute of Standards and Technology Mass Spectral database (NIST-MS). The percentage of each component was calculated from relative peak area of each component in the chromatogram.

The identified 54 bioactive compounds from *P.nigrum* and its 2D structure were retrieved from Chemspider database (http://www.chemspider.com/).The above said 2D structures were converted in to 3D structure using swisspdb viewer (http:// www.spdbv.vital-it.ch/). Aerolysin (aerA) is retrieved from UniProtKB/Swiss-Prot database (http://www.uniprot.org/). Aerolysin (aerA)is treated with these bioactive compounds using Hex docking Software (hex.loria.fr/dist50/).

RESULTS

Gas Chromatography – Mass Spectroscopy (GC-MS) analysis

P.nigrum contains 54 bioactive compounds which was identified from GC-MS analysis and NIST library (Table.1). The GC-MS chromatogram of *P.nigrum* is shown in Figure 2. All these compounds were pharmacologically important and they showed the properties such as analgesic, antidiabetic, antibacterial and antifungal activity.

Molecular docking

In order to find out the best effective drug, docking was carried out with help of Hex software. In this docking, Acetic acid, 1-methylethyl ester, Methylglyoxal, 2,3-Butanediol, [S-(R*,R*)]-à-Phellandrene, 2-Cyclopenten-1-one, 2-hydroxy-, Bicyclo[3.1.0]hex-2-ene, 4-methyl-1-(1-methylethyl)-, 1-Piperidinee thanamine, Cyclo hexene, 4-methyl-1-(1-methylethenyl)-, Bicyclo [3.1.0]hexan-2-ol, 2-methyl-5-(1-methylethyl)-, (1à,2à,5à)-, dl-Malic disodium salt, 1,6-Octadien-3-ol, 3,7-dimethyl-, Terpineol, cis-á-, 1-Piperidinecarboxaldehyde, 3-Cyclohexen -1-ol, 4-methyl-1-(1-methylethyl)-, 3-Cyclohexene -1-methanol, à,à4-trimethyl-, 4-Thujen-2à-yl acetate, Limonene oxide, trans-, 3-Nonanol, 1,2;6,7-diepoxy-3,7-dimethyl-, acetate, 1-Piperidineethanamine, 1,3-Benzodioxole, 5-(2-propenyl)-, 2-Methoxy-4-vinylphenol, Cyclohexene, 4-ethenyl-4-methyl-3-(1-methylethenyl)-1-(1-methylethyl)-, (3R-trans)-, Eugenol, Copaene, Bicyclo[5.3.0]decane, 2-methylene-5-(1-methylvinyl)-8-methyl-, Bicyclo[3.1.1]hept-2-ene, 2,6-dimethyl-6-(4-methyl-3-pentenyl)-, Bicyclo[7.2.0]undec-4-ene, 4,11,11-trimethyl-8-methylene-,[1R-(1R*,4Z,9S*)]-, 1,6,10-Dodecatriene, 7,11-dimethyl-3-methylene-, à-Caryophyllene, Benzene, 1-(1,5-dimethyl-4-hexenyl)-4-methyl-, 1,6-Cyclodecadiene, 1-methyl-5-methylene-8-(1-methylethyl)-, [s-(E,E)]-, Eudesma-4(14),11-diene, Cyclohexene, 1-methyl-4-(5-methyl-1-methylene-4-hexenyl)-, (S)-, á-Bisabolene, Naphthalene, 1,2,3,5,6,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl)-, (1S-cis)-, 1-Hydroxy-1,7-dimethyl-4-isopropyl-2,7-cyclodecadiene, Cyclohexene, 4-(1,5-dimethyl-1,4-hexadienyl)-1-methyl-, 3,7-Cyclodecadiene-1-methanol, à,à,4,8-tetramethyl-, [s-(Z,Z)], Bicyclo[4.3.0]nonane, 7-methylene-2,4,4-trimethyl-2-vinyl-, (-)-Spathulenol, Caryophyllene oxide, 1-Naphthalenol, 1,2,3,4,4a,7,8,8a-octahydro-1,6-dimethyl-4-(1-methylethyl)-, [1R-(1à,4á,4aá,8aá)]-, 2-Naphthalenemethanol, decahydro-à,à,4a-trimethyl-8-methylene-, [2R-(2à,4aà,8aá)]-, Levomenol, 2-Octenoic acid, 4-isopropylidene-7-methyl-6-methylene-, methyl ester, 9H-Pyrido[3,4-b]indole, 8-hydroxy-1-methyl-, Bicyclo[3.1.1]hept-3-ene, 2-formylmethyl-4,6,6-trimethyl-, 2-Naphthalenemethanol, 2,3,4,4a,5,6,7,8-octahydro-à,à,4a,8-tetramethyl-, [2R-(2à,4aá,8á)]-, Naphthalene, decahydro-, Piperidine, 1-(1-oxo-3-phenyl-2-propenyl)-, 2-

Hydroxy-2-phenylbutyramide, Benzo[b] cyclopenta [e]pyrane-3-carboxaldehyde, 1,2-dihydro-, Furane-2-carbohydrazide, 5-phenylethynyl-, o-Anisic acid, 2-adamantyl ester and Piperine from *Piper nigrum* is selected as bioactive compounds which were docked against virulent factor Aerolysin (aerA) (Fig.1)from *A.hydrophila*is .These ligands have been used to target against Aerolysin (aerA) which bound to the receptor to inhibit its function. The length of the receptor is 940 amino acids (Fig.2).The nature of the complex between the drug and the receptor molecule was identified via docking and the inhibition nature of the ligands and their binding affinities were calculated using free energy simulations. Docking results between Aerolysin (aerA) receptor and phytochemical drugs were tabulated (Table.1). In this study, Piperine (Fig.1) from *Piper nigrum* showed a maximum e-value -698.77 (Fig.3).

Table 1. shown molecular docking of fish disease causing aerolysin (aer A) against bioactive compounds Qualitative determination of biochemical constituents in *Piper nigrum* by GC-MS

S.No.	Peak name	Retention time	Receptor Name	e-values
1.	Acetic acid, 1-methylethyl ester Formula: $C_5H_{10}O_2$ MW: 102	3.28	Aerolysin (aer A)	-174.54
2.	Methylglyoxal Formula: $C_3H_4O_2$ MW: 72	4.06	Aerolysin (aer A)	-165.60
3.	2,3-Butanediol, [S-(R*,R*)]- Formula: $C_4H_{10}O_2$ MW: 90	4.21	Aerolysin (aer A)	-150.42
4.	à-Phellandrene Formula: $C_{10}H_{16}$ MW: 136	6.38	Aerolysin (aer A)	-171.38
5.	2-Cyclopenten-1-one, 2-hydroxy- Formula: $C_5H_6O_2$ MW: 98	6.53	Aerolysin (aer A)	-182.92
6.	Bicyclo[3.1.0]hex-2-ene, 4-methyl-1-(1-methylethyl)- Formula: $C_{10}H_{16}$ MW: 136	7.32	Aerolysin (aer A)	-346.12
7.	1-Piperidineethanamine Formula: $C_7H_{16}N_2$ MW: 128	8.42	Aerolysin (aer A)	-364.54
8.	Name: Cyclohexene, 4-methyl-1-(1-methylethenyl)- Formula: $C_{10}H_{16}$ MW: 136	8.98	Aerolysin (aer A)	-263.71
9.	Name: Bicyclo[3.1.0]hexan-2-ol, 2-methyl-5-(1-methylethyl)-, (1à,2à,5à)- Formula: $C_{10}H_{18}O$ MW: 154	9.31	Aerolysin (aer A)	-332.49
10.	dl-Malic disodium salt Formula: $C_4H_6O_5$ MW: 134	9.41	Aerolysin (aer A)	-100.51
11.	1,6-Octadien-3-ol, 3,7-dimethyl- Formula: $C_{10}H_{18}O$ MW: 154	9.79	Aerolysin (aer A)	-112.43
12.	Terpineol, cis-á- Formula: $C_{10}H_{18}O$	9.94	Aerolysin (aer A)	-210.39

	MW: 154			
13.	Name: 1-Piperidinecarboxaldehyde Formula: $C_6H_{11}NO$ MW: 113	10.83	Aerolysin (aer A)	-182.44
14.	Name: 3-Cyclohexen-1-ol, 4-methyl-1-(1-methylethyl)- Formula: $C_{10}H_{18}O$ MW: 154	11.48	Aerolysin (aer A)	-213.26
15.	Name: 3-Cyclohexene-1-methanol, à,à4-trimethyl- Formula: $C_{10}H_{18}O$ MW: 154	11.75	Aerolysin (aer A)	-278.54
16.	Name: 4-Thujen-2à-yl acetate Formula: $C_{12}H_{18}O_2$ MW: 194	11.95	Aerolysin (aer A)	-270.30
17.	Name: Limonene oxide, trans- Formula: $C_{10}H_{16}O$ MW: 152	12.50	Aerolysin (aer A)	-264.71
18.	Name: 3-Nonanol, 1,2;6,7-diepoxy-3,7-dimethyl-, acetate Formula: $C_{13}H_{22}O_4$ MW: 242	13.12	Aerolysin (aer A)	-250.40
19.	Name: 1-Piperidineethanamine Formula: $C_7H_{16}N_2$ MW: 128	13.27	Aerolysin (aer A)	-90.83
20.	Name: 1,3-Benzodioxole, 5-(2-propenyl)- Formula: $C_{10}H_{10}O_2$ MW: 162	13.50	Aerolysin (aer A)	-243.17
21.	Name: 2-Methoxy-4-vinylphenol Formula: $C_9H_{10}O_2$ MW: 150	13.90	Aerolysin (aer A)	-237.70
22.	Name: Cyclohexene, 4-ethenyl-4-methyl-3-(1-methylethenyl)-1-(1-methylethyl)-, (3R-trans)- Formula: $C_{15}H_{24}$ MW: 204	14.17	Aerolysin (aer A)	-13.64
23.	Name: Eugenol Formula: $C_{10}H_{12}O_2$ MW: 164	14.59	Aerolysin (aer A)	-256.25
24.	Name: Copaene Formula: $C_{15}H_{24}$ MW: 204	14.94	Aerolysin (aer A)	-218.09
25.	Name: Bicyclo[5.3.0]decane, 2-methylene-5-(1-methylvinyl)-8-methyl- Formula: $C_{15}H_{24}$ MW: 204	15.13	Aerolysin (aer A)	-19.92
26.	Name: Bicyclo[3.1.1]hept-2-ene, 2,6-dimethyl-6-(4-methyl-3-pentenyl)- Formula: $C_{15}H_{24}$ MW: 204	15.49	Aerolysin (aer A)	-234.35
27.	Name: Bicyclo[7.2.0]undec-4-ene, 4,11,11-trimethyl-8-methylene-,[1R-(1R*,4Z,9S*)]-	15.76	Aerolysin (aer A)	-221.80

	Formula: $C_{15}H_{24}$ MW: 204			
28.	Name: 1,6,10-Dodecatriene, 7,11-dimethyl-3-methylene- Formula: $C_{15}H_{24}$ MW: 204	16.00	Aerolysin (aer A)	-196.63
29.	Name: à-Caryophyllene Formula: $C_{15}H_{24}$ MW: 204	16.35	Aerolysin (aer A)	-255.51
30.	Name: Benzene, 1-(1,5-dimethyl-4-hexenyl)-4-methyl- Formula: $C_{15}H_{22}$ MW: 202	16.58	Aerolysin (aer A)	-143.42
31.	Name: 1,6-Cyclodecadiene, 1-methyl-5-methylene-8-(1-methylethyl)-, [s-(E,E)]- Formula: $C_{15}H_{24}$ MW: 204	16.74	Aerolysin (aer A)	-150.03
32.	Eudesma-4(14),11-diene Formula: $C_{15}H_{24}$ MW: 204	16.89	Aerolysin (aer A)	-161.86
33.	Cyclohexene, 1-methyl-4-(5-methyl-1-methylene-4-hexenyl)-, (S)- Formula: $C_{15}H_{24}$ MW: 204 á-Bisabolene	16.98	Aerolysin (aer A)	-148.81
34.	Naphthalene, 1,2,3,5,6,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl)-, (1S-cis)- Formula: $C_{15}H_{24}$ MW: 204	17.24	Aerolysin (aer A)	-160.68
35.	1-Hydroxy-1,7-dimethyl-4-isopropyl-2,7-cyclodecadiene Formula: $C_{15}H_{26}O$ MW: 222	17.30	Aerolysin (aer A)	-260.82
36.	Name: Cyclohexene, 4-(1,5-dimethyl-1,4-hexadienyl)-1-methyl- Formula: $C_{15}H_{24}$ MW: 204	17.47	Aerolysin (aer A)	-248.77
37.	Name: 3,7-Cyclodecadiene-1-methanol, à,à,4,8-tetramethyl-, [s-(Z,Z)] Formula: $C_{15}H_{26}O$ MW: 222	17.78	Aerolysin (aer A)	-227.39
38.	Name: Bicyclo[4.3.0]nonane, 7-methylene-2,4,4-trimethyl-2-vinyl- Formula: $C_{15}H_{24}$ MW: 204	18.27	Aerolysin (aer A)	-244.79
39.	Name: (-)-Spathulenol Formula: $C_{15}H_{24}O$ MW: 220	18.33	Aerolysin (aer A)	-255.83
40.	Name: Caryophyllene oxide Formula: $C_{15}H_{24}O$ MW: 220	18.42	Aerolysin (aer A)	-143.42
41.	Name: 1-Naphthalenol, 1,2,3,4,4a,7,8,8a-	19.29	Aerolysin	-150.03

	octahydro-1,6-dimethyl-4-(1-methylethyl)-, [1R-(1à,4á,4aá,8aá)]- Formula: $C_{15}H_{26}O$ MW: 222		(aer A)	
42.	Name: 2-Naphthalenemethanol, decahydro-à,à,4a-trimethyl-8-methylene-, [2R-(2à,4aà,8aá)]- Formula: $C_{15}H_{26}O$ MW: 222	19.51	Aerolysin (aer A)	-148.81
43.	Name: Levomenol Formula: $C_{15}H_{26}O$ MW: 222	19.78	Aerolysin (aer A)	-160.68
44.	Name: 2-Octenoic acid, 4-isopropylidene-7-methyl-6-methylene-, methyl ester Formula: $C_{14}H_{22}O_2$ MW: 222	20.70	Aerolysin (aer A)	-161.86
45.	Name: 9H-Pyrido[3,4-b]indole, 8-hydroxy-1-methyl- Formula: $C_{12}H_{10}N_2O$ MW: 198	20.99	Aerolysin (aer A)	-154.50
46.	Name: Bicyclo[3.1.1]hept-3-ene, 2-formylmethyl-4,6,6-trimethyl- Formula: $C_{12}H_{18}O$ MW: 178	21.16	Aerolysin (aer A)	-238.70
47.	Name: 2-Naphthalenemethanol, 2,3,4,4a,5,6,7,8-octahydro-à,à,4a,8-tetramethyl-, [2R-(2à,4aá,8á)]- Formula: $C_{15}H_{26}O$ MW: 222	21.87	Aerolysin (aer A)	-281.51
48.	Name: Naphthalene, decahydro- Formula: $C_{10}H_{18}$ MW: 138	23.44	Aerolysin (aer A)	-151.38
49.	Name: Piperidine, 1-(1-oxo-3-phenyl-2-propenyl)- Formula: $C_{14}H_{17}NO$ MW: 215	27.74	Aerolysin (aer A)	-243.05
50.	Name: 2-Hydroxy-2-phenylbutyramide Formula: $C_{10}H_{13}NO_2$ MW: 179	32.96	Aerolysin (aer A)	-140.11
51.	Name: Benzo[b]cyclopenta[e]pyrane-3-carboxaldehyde, 1,2-dihydro- Formula: $C_{13}H_{10}O_2$ MW: 198	35.75	Aerolysin (aer A)	-220.11
52.	Name: Furane-2-carbohydrazide, 5-phenylethynyl- Formula: $C_{13}H_{10}N_2O_2$ MW: 226	35.95	Aerolysin (aer A)	-264.85
53.	Name: o-Anisic acid, 2-adamantyl ester Formula: $C_{18}H_{22}O_3$ MW: 286	36.22	Aerolysin (aer A)	-211.14

54.	Name: Piperine Formula: $C_{17}H_{19}NO_3$ MW: 285	36.64	Aerolysin (aer A)	**-698.77**

Figure 1. Structure of Piperine

Figure 2. Molecular Structure ofaerolysin (aer A)

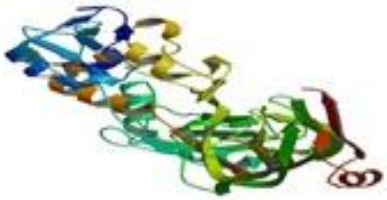
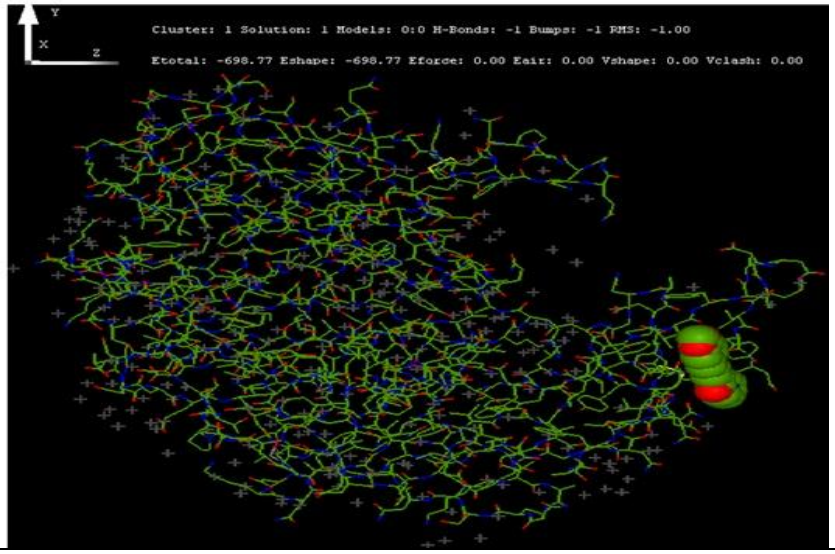


Figure 3. Molecular docking of aerolysin (aer A) with Piperine



DISCUSSION

A.hydrophila is a gram-negative, bacterial pathogen causing fish disease. The severity of disease may be dependent on expression of specific virulence factors, which vary among strains called pathotypes. Therefore, it is very important to distinguish highly virulent *A. hydrophila* pathotypes from those less virulent for disease control. *A. hydrophila* pathogenesis identified presence of known virulence factor aerolysin (aerA). *Aeromonas hydrophila* from ornamental fish sources produced particularly aerA genes, responsible for aerolysin toxin production. Antibiotics such as chloramphenicol, florenicol, tetracycline, sulfonamide, nitrofuran derivatives, and pyridine carboxylic acids are used to eliminate and control the infection of *A. hydrophila*. Terramycin is placed in fish food during hatchery operations as another chemotherapeutic agent in preventing *A. hydrophila* [2]. The occurrence of antibiotic resistant bacteria associated with fish diseases is a worldwide problem in aquaculture, which has received considerable attention in the last years and this issue continues to increase due to the absence of a more effective and safer use of antibiotics [9]. Chemotherapy has progressed internationally for treating the most diversified infectious disease of fish [10]. However, there are problems associated with the use of such chemicals. It was the demand of the time to look for alternative means of commercial synthetic drugs. Medicinal plants are vital source of drugs from the ancient time holding the scenario of the Indian system of medicine [11]. According to Ghani medicinal plants are rich sources of bioactive compounds and thus serve as important raw materials for drug production [12]. Antibiotics used in medicines have been tried experimentally to treat bacterial infections of fish. Problems including solubility, palatability, toxicity, cost, delivery and governmental restrictions have limited the available antibiotics to a select few, especially in ornamental fish culture [9]. Increasing failures in antibiotic resistance exhibited by microbial pathogens has led to screening of several medicinal plants for their potential antimicrobial activity [13-16]. Traditionally, black pepper has been used in a variety of different remedies and for different purposes. According to Ayurveda, the pungency

and heating properties of black pepper work to help metabolize food as it is digested in our system. Its heat works as a stimulant like lighting a fire might [17]. This stimulating quality is also used to clear congestion in the respiratory system as well as other processes.

P.nigrum is one of the most widely used among spices. It is valued for its distinct biting quality attributed to the alkaloid, piperine. Black pepper is used not only in human dietaries but also for a variety of other purposes such as medicinal, as a preservative, and in perfumery. Many physiological effects of black pepper, its extracts, or its major active principle, piperine, have been reported in recent decades. Piperine, along with its isomer chavicine, is the alkaloid responsible for the pungency of black pepper and long pepper. It has also been used in some forms of traditional medicine and as an insecticide [18].

Use of herbal medicinal preparations as antimicrobial agents to combat this situation can become a sustainable solution for this problem. These traditional systems of health care and longevity [19] are still being rejected by many due to a lack of standardization of the procedures and methods of their use [20].

Based on previous reports Prabhu and Guruvayoorappan states that levels of 10, 20 and 40 mg/kg of mangrove extract was incorporated in to feed for measuring its effect on the immune response and disease resistance of clownfish. Molecules derived from natural products have had an excellent record of providing novel chemical structures for the development of new therapeutic agents. Many of the world's most valuable and successful medicines have been derived from sources in nature. An antimicrobial agent originating from marine halophytes is an immediate necessity in developing novel marine pharmaceuticals. Literature on antibacterial studies of natural product on fish pathogens is comparatively rare [21].

In the present study *P.nigrum* taken into consideration. These plants produced bioactive compounds tested against fish disease causing receptor through molecular docking. Based on the energy values best bioactive compound is identified as drug molecule. Among 54 bioactive compounds Piperine from *P.nigrum* showed a maximum e-value -698.77.

CONCLUSION

A.hydrophila contains aerA genes which produced aerolysin toxin production and responsible for fish diseases. *P.nigrum* is one of the most widely used medicinal plants. It is valued for its distinct biting quality attributed to the alkaloid, piperine. *P.nigrum* is used not only in human dietaries but also fish disease curing agent. In that plant produces number of bioactive compounds, piperine is the best ideal compound for curing fish disease. In the present study aerolysin (aerA) is docked against 54 bioactive compounds from *P.nigrum.* Out of 54 bioactive compounds Piperine produce high energy negative value -698.77 through molecular docking. From the above study it is concluded that Piperine is identified as best ideal compound for *A.hydrophila* causing fish disease.

ACKNOWLEDGEMENT: None

CONFLICT OF INTEREST:
The authors declare that they have no conflict of interest.

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