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Research Article

Evaluation of Bacterial Infections on Caudal Fin Regeneration in Zebrafish and Effect of Restorative Plants

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ABSTRACT

In this present work to exploring how the recovery limit in the time of the caudal balance recovery of ordinary and bacterial disease (*Pseudomonas aeruginosa*, *Flexibacter columnaris* and *Aeromonas hydrophila*) in zebra fish and relative examination of focusing on tainting microorganisms by the the plants (*Azadirachta indica*, *Moringa oleifera* and *Cynodon dactylon*) extracts and antimicrobials. Intraperitoneal infusion and water dissolving strategy were utilizing for bacterial disease in zebrafish followed by standard protocol. Four distinctive plant extracts utilizing for biocidal movement. In the intraperitoneal injection method, the fish was not live. Typical caudal fin recovery begins gradually at a time augments and accomplishes, it is a full recovery in twelfth days. *A. hydrophila* and *P. aeruginosa* those microorganisms were making contaminating to zebrafish and it doesn't have noteworthy recovery prepare in water dissolve method. However, *F. columnaris* is not contaminated the zebrafish and it discovered ordinary recovery process were watched. The plant extracts of *A. indica* accepted more opportunity to solution the fish malady, even chloramphenicol cured (ten days) the illness in brief period and the caudal fin recovery handle recaptured. In comparing to chloroform extract of *A. indica* showed maximum activity in order to *F. columnaris* (6±0.3 mm), *A. hydrophila* (5±0.25 mm), *A. hydrophila* and *P. aeruginosa* (4±0.2 mm). Presence of the bacteria in the infected cells might lead to the inhibition of regeneration in the amputated region and chloroform extract of *Azadirachta indica* could be utilized for the advancement of new sorts of antibacterial specialists and may hence be utilized as remedial in caudal fin recovery of zebra fish.

Keywords*:* Zebra fish, Caudal fin, Regeneration, Antibacterial activity.

INTRODUCTION

In science, regeneration is the procedure of development over again of lost tissue or obliterated parts or organs. In any case, primarily alludes to the morphogenic forms that describe the phenotypic pliancy of attributes permitting multicellular living beings to repair and keep up the respectability of their physiological and morphological states[1]. Biological systems are regenerative also, an unsettling influence, for example, a fire of bug episode in a timberland, spearheading animal varieties will involve, vie for space, and set up themselves in the recently opened natural surroundings. The new development of seedlings and group gathering procedure is known as regeneration in nature[2]. The zebra fish is a generally utilized model creature to concentrate the recovery of organs, for example, the blade[3], nerve tissues[4] and heart[5,6]. Jazwinska trusts that the human body may have, after some time, essentially lost the capacity to trigger recovery it is conceivable that regenerative operators exist in the human body. However, recovery is restricted due to the loss of planning components or as a result of constraint by over proficient scarring[7]. In creatures with regenerative capacity, defensive layers of tissue cover the site of harm and the cells underneath then change into begetter or undifferentiated organisms, shaping a development zone named a blastema. In people and other higher creatures, the capacity to deliver foundational microorganisms along these lines is absent. In this manner, understanding the hereditary programming and flagging instruments by which blastemas are shaped is major for setting up such capacity in people, and it is these that are the principle center of research[8]. Transgenesis lines amid zebrafish heart recovery unmistakably ensnare a save ancestor cell and focuses the path for future tests that ought to be intended to find the source of the blastemal, as of late[9]. Strangely, grown-up zebrafish epicardial cells hold this plastic conduct. Maybe the most striking part of the zebrafish epicardium amid recovery is that it experiences a to a great degree quick and element reaction to damage[5,10]. Treatment with restorative plants having antibacterial action is a conceivably gainful option in the aquaculture. As of late research has been started to assess the plausibility of homegrown medications in fish illnesses[11]. In this present study to investigating the regeneration limit in the time of zebra fish caudal fin recovery in typical and bacterial disease and a similar examination of focusing on tainting microscopic organisms by plant concentrates and antimicrobials.

MATERIALS AND METHODS

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Fish stock, maintenance, staging and caudal fin observation

Adult zebrafish were maintained at room temperature (28 ± 0.5 ºC). Fish that were 3-14 months old were used for regeneration experiments. Caudal fins were amputated along the dorsoventrally axis, intersecting the median rays approximately halfway. Fish were allowed to regenerate at 28 ± 0.5 ºC in maintained tank[12].

Caudal fin regeneration of Zebra fish

Intraperitoneal injection and water dissolving method were employing for bacterial infection in zebrafish by *A. hydrophila*, *P. aeruginosa* and *F. columnaris*. In intraperitoneal injection method, harvested bacterial stains were washed twice with sterile phosphate-buffered saline (PBS), and then resuspended in PBS to appropriate concentrations. Fishes were divided into three groups, each group containing five fish. In intraperitoneally injected method 0.3µl of serially tenfold diluted bacterial suspensions containing 10^1 - 10^8 CFU. In positive control, injected intraperitoneally with 0.3 µl sterile PBS. All experimental fishes are maintained were separate tank[13]. In water dissolving method**,** 500 ml of water in each three glass tanks. 50µl of three different bacterial strains were inoculated in each glass bottles and incubated for 1 weak and observe the regeneration process in the caudal fin.

Preparation of plant extraction

Fresh leaf was cut into small pieces and shocked with acetone for Büchner funnel a period of 48 h, separately. The extract was filtered through Büchner funnel with Whatman No.1 filter paper. The filtrate samples were dried by using rotary evaporator. The evaporated samples were stored in the refrigerator and used for further uses[14].

Bacterial susceptibility testing

The antimicrobial activity was tested by the well diffusion method according to National Committee for Clinical Laboratory Standards (NCCLS) [15]. Petri plates containing 20 ml of Nutrient Agar medium were seeded with 24 h cultures of bacterial inoculums (a standardized inoculums 1-2 x 10 7 cfu/ml 0.5 Mcfarland standard). Wells (6 mm diameter) were cut into the agar and 50 µl of plant extracts were tested in a concentration of 100 mg/ml and incubation was performed at 37 ºC for 24 h.

RESULTS

Caudal fin regeneration of normal zebra fish

Zebra fish was sedated and amputation was made in caudal fin region. There are no changes was observed in the first day, from the second day on words regeneration was observed (Figure 1). Regeneration starts very slowly but gradually increases and attains its full regeneration in 12th days. Figure 2 shows the rate of complete regeneration. After that pigment started to appear from the anterior region to posterior region within fifteen days from the day of amputation. During regeneration, male character was not expressed (length of the lower part of caudal fin of male fish is longer than female), it grows to the normal length.

Caudal fin regeneration of zebra fish by bacterial infection

There are two methods were followed in the intraperitoneal injection method the fish not live. It is death after few hours. Then selected the water dissolving method. In this method, the fish were affected within 25 days and fin regeneration was stopped (Figure 3). *A. hydrophila* and *P. aeruginosa* these two bacteria is infecting the zebrafish and stop the regeneration process. But the *F. columnaris* is not infected the zebrafish so that the normal regeneration process started. Figure 4, monograph mention the three different pathogen regeneration within 25 days of acute treatment.

Caudal fin regeneration of zebra fish by Chloramphenicol Antibiotic and Azadirachta indica extract

The disease was cure day by day within ten days the disease is decrease and the regeneration process was started again. Then after towel days, the zebrafish caudal fin is fully regenerated.

A. indica and Chloramphenicol were used for curing the bacterial skin disease in zebrafish. Administration of Chloramphenicol in the infected fish water resulted in the curing of the skin disease within 10 days and helped in the caudal fin regeneration (Figure 5). The plant extract *A. indica* took more time to cure the fish disease but Chloramphenicol cured the disease in short period and the caudal fin regeneration process regained (Figure 6).

Antibacterial activity of medicinal plants

Acetone extract of *A. indica* showed maximum (5±0.25 mm) activity against *A. hydrophila*, minimum activity against *P. aeruginosa* (2.5±0.05 mm) and *F. columnaris* (1.8±0.05 mm). Chloroform extract *A. indica* showed maximum *F. columnaris* (6±0.3 mm), minimum (5±0.25 mm) activity against *A. hydrophila* and *P. aeruginosa* (4±0.2 mm). Ethyl acetate extract of *A. indica* showed maximum (4±0.2 mm) activity against from *A. hydrophila* and *P. aeruginosa*, and minimum (3±0.15 mm) activity against *F. columnaris* (Figure 7 A). Acetone extract of *M. oleifera* showed maximum (5mm) activity against *A. hydrophila*, Minimum (2±0.1 mm) activity against *P. aeruginosa* and *F. columnaris* (1±0.05 mm). Chloroform extract of *M. oleifera* showed maximum (4±0.2 mm) *A. hydrophila* minimum (2±0.1 mm) activity against *P. aeruginosa*. Ethyl acetate extract of *M. oleifera* showed maximum (4±0.2 mm) activity against *A. hydrophila*. There is no significant activity against *Pseudomonas aeruginosa, F. columnaris* (Figure 7 B). Acetone extract of *C. dactylon* showed no activity against *A. hydrophila*, *P. aeruginosa*, and *F. columnaris*. Chloroform extract of *C. dactylon* showed no activity against *A. hydrophila, P. aeruginosa*, minimum (1±0.05 mm) activity against from *F. columnaris*. Ethyl acetate extract of *C. dactylon* showed maximum (2±0.1 mm) activity against *F. columnaris* and minimum (1mm) activity against from *P. aeruginosa* and *A. hydrophila* (Figure 7 C). Water extract of all three plants extracts showed no significant activity against from all pathogens (Figure 7).

Effective plant extract compare with antibiotic

Chloramphenicol antibiotic is high effective compared to the other antibiotics and *A. indica* is high effective compared to other two medicinal plants. Chloramphenicol and *A. indica* are compare which one is high effective that is used for the cure the bacterial infection in zebrafish (Figure 8). Chloramphenicol shows the maximum (9±0.45

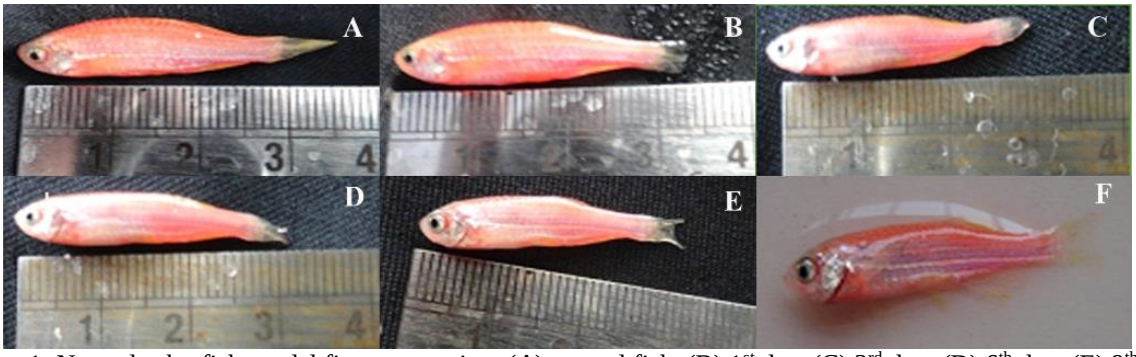

Figure 1: Normal zebrafish caudal fin regeneration. (A) control fish, (B) 1st day, (C) 3rd day, (D) 6th day, (E) 9th day and (F) 12th day.

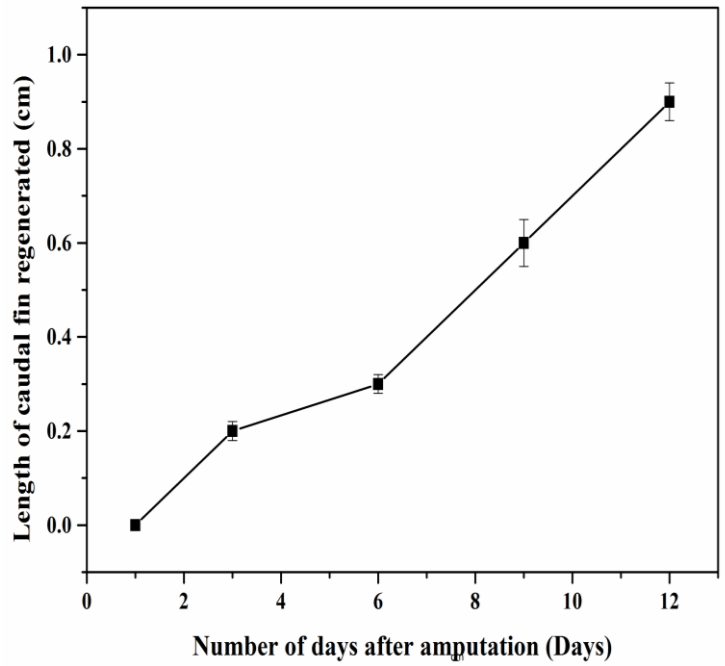

Figure 2: Graphical representation shows the regeneration in caudal fin of normal zebrafish.

mm) activity *A. hydrophila*, (6±0.3 mm) activity against *P. aeruginosa* and minimum activity from *F. columnaris* (5±0.25 mm). *A. indica* show the Maximum (4±0.2 mm) activity against *Pseudomonas aeruginosa*, Minimum activity against from *F. columnaris* and *A. hydrophila* (3±0.15 mm). Chloramphenicol has high activity against bacterial pathogens.

DISCUSSION

Regeneration of caudal fin in zebrafish are the most projected regeneration studies among vertebrates. Zebra fish are known for their ability to regenerate spinal cord, fin, and retinal tissue, but their capacity for cardiac regeneration has not been evaluated[16]. Genes (*wnt, hox, fgf, fgfr* and *msx*) are shown to be differentially expressed for controlling the regeneration mechanism, without understanding a defiant pathway and biomechanism for the regeneration[6]. When a zebrafish loses its fin, a special group of cells forms on the remaining stump (these cells may be "pluripotent" stem cells). These cells, which appear identical to one another, regrow the entire limb, complete with all cell types required for a complex organ[17].

We hypothesized that the presence of the bacteria in the infected cells might lead to the inhibition of regeneration in the amputated region (Figure 1), while these regions were well known as the province of stem cells (blastema). Bacterial-mediated suppression of stem cell replication might be a reason for this kind of depletion of the stem cell population (Figure 3). Quiescent human hematopoietic Stem Cells (HSCs) are showing resistance to infection by the intracellular and extracellular pathogens *Listeria monocytogenes* and *Yersinia enterocolitica*[18]. Our hypothesis is supported by a recent report by Wang (2013), the suppression of Intestine Stem Cell (ISC) division by *Vibrio cholerae* may be a strategy to enhance its colonization in the epithelial surface and its principal virulence factor CTX might suppress ISC division. But, the mode how this bacterium affects the stem cell population in the amputated and wound region of the skin is unknown. Preferential infection may be a mode which the bacteria lead to the reduction in the stem cell population (Figure 3)[19]. Ermin (2010) has reported on bacterial dermato-septicemia, a systemic infectious bacterial disease of frogs can be caused by several opportunistic gram-negative bacterial species including *A. hydrophila*, *C. indologenes*,

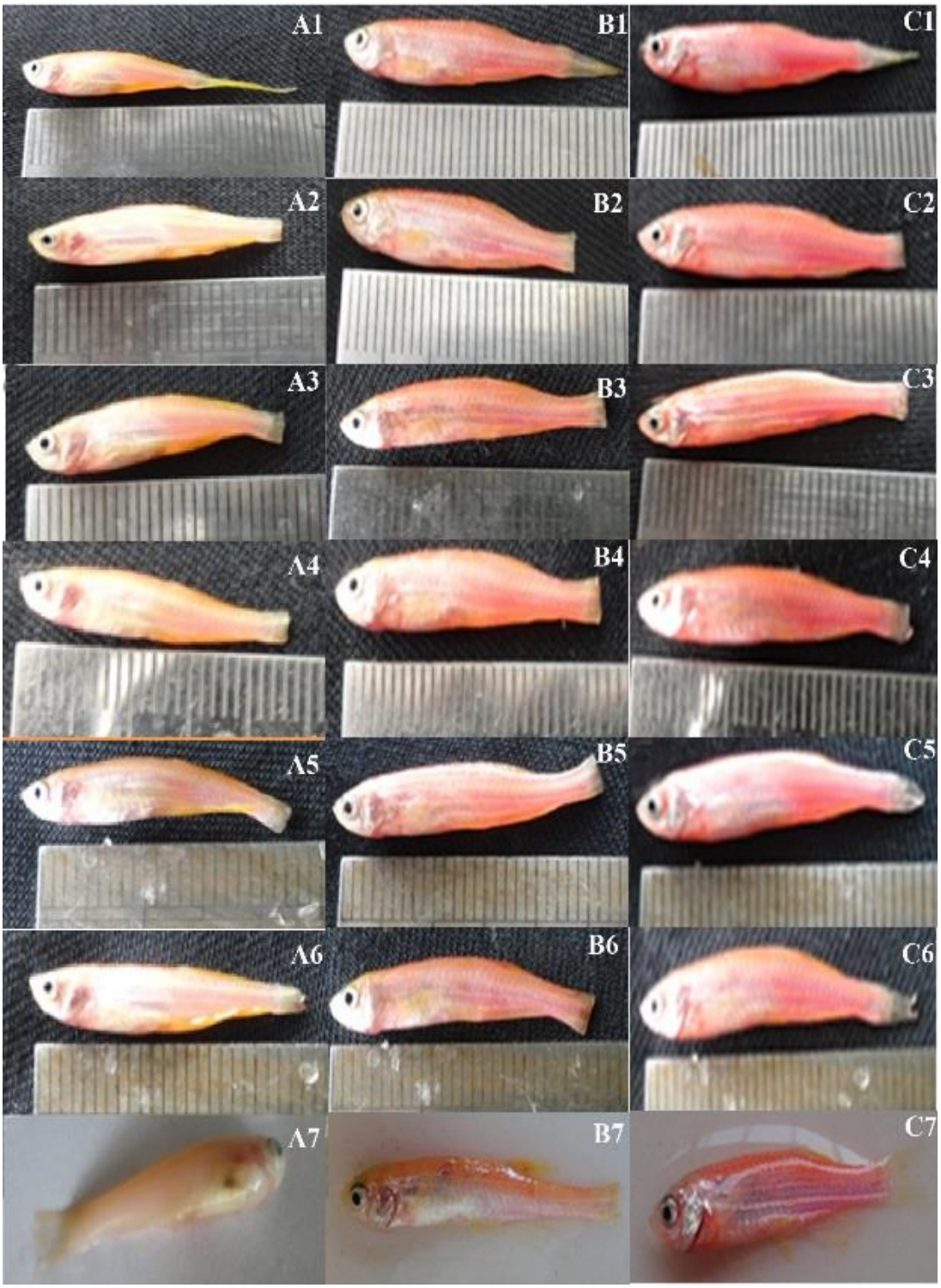

Figure 3: *Aeromonas hydrophila* infection in zebrafish, halted the caudal fin regeneration. A1 – A7 represented as *A. hydrophila*, B1 – B7 represented as *P. aeruginosa* and C1 – C7 represented as *F. columnaris.*

C. meningosepticum, C. freundii, K. pneumoniae, P. mirabilis, P. aeruginosa, and *S. liquifaciens*[20]. Here we determined the pathogenicity of three bacterial species (*A. hydrophila, P. aerugenosa,* and *F. columnaries)* are used for the experiments in zebra fish. Morbidity and mortality were 40% of *F. columnaris* and 20 % of *A. hydrophila*, and the organism was reisolated from the skin of affected animals (Not reported).

Ryan (2010) stated on his work on Cystic fibrosis (CF) is a genetic disease caused by recessive mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) gene and is associated with prevalent and chronic *Pseudomonas aeruginosa* lung infections[21]. The present work highlights the zebrafish as a powerful model organism for human infectious disease, particular infection by *P. aeruginosa*. Zebrafish embryos with reduced

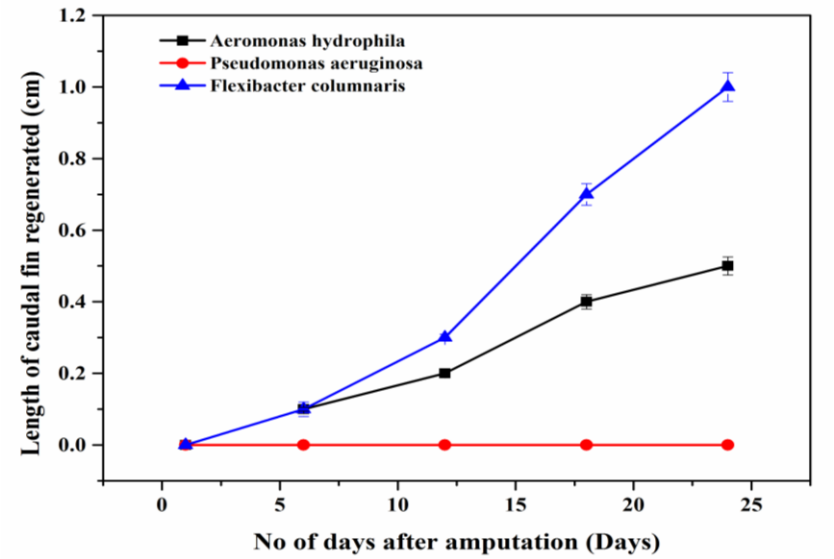

Figure 4: *Aeromonas hydrophila*, *Pseudomonas aeruginosa* and *Flexibacter columnaris* infected in zebrafish caudal fin regeneration.

Figure 5: Infected fish treated with Antibiotics (Chloramphenicol) caudal fin normally regenerated. Infected fish treated with an antibiotic (A), 5th day after treated (B), 8th day after treated (C), 10th day after treated (D), 15th day after treated (E), full recreation (F).

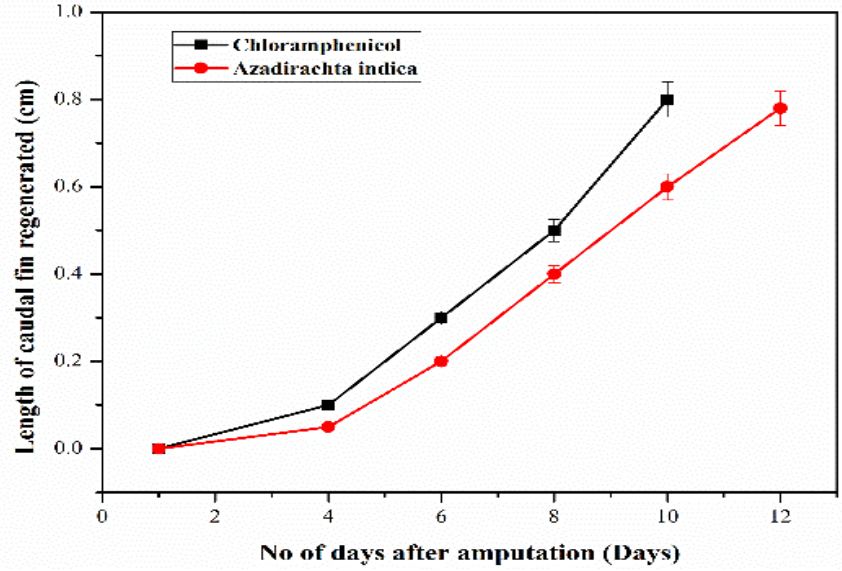

Figure 6: Monograph shows the caudal fin reaeration by treating *A. indica* plant extract and Chloramphenicol.

expression of the *cftr* gene (*cftr* morphants) exhibited reduced respiratory burst response and directed neutrophil migration, supporting a connection between *cftr* and the innate immune response. Characteristics of this organism that distinguish it from other, apparently nonpathogenic, gliding bacteria of fish origin include its strongly adherent and rhizoid, yellow, flat colonies on solid media; its absorption of Congo red dye, and its production of Flexi Rubin-type pigments. In order to access the *A. hydrophila*, *P. aerugenosa*, and *F. columnaries* were tested for their using the medicinal plants *A. indica*, *C. dactylon*, and *M. oleifera*[22].

CONCLUSION

The caudal fin is one of the most convenient tissues to approach experimentally due to its accessibility, simple structure, and fast regeneration. In this work, we investigate the regeneration capacity of the zebra fish is

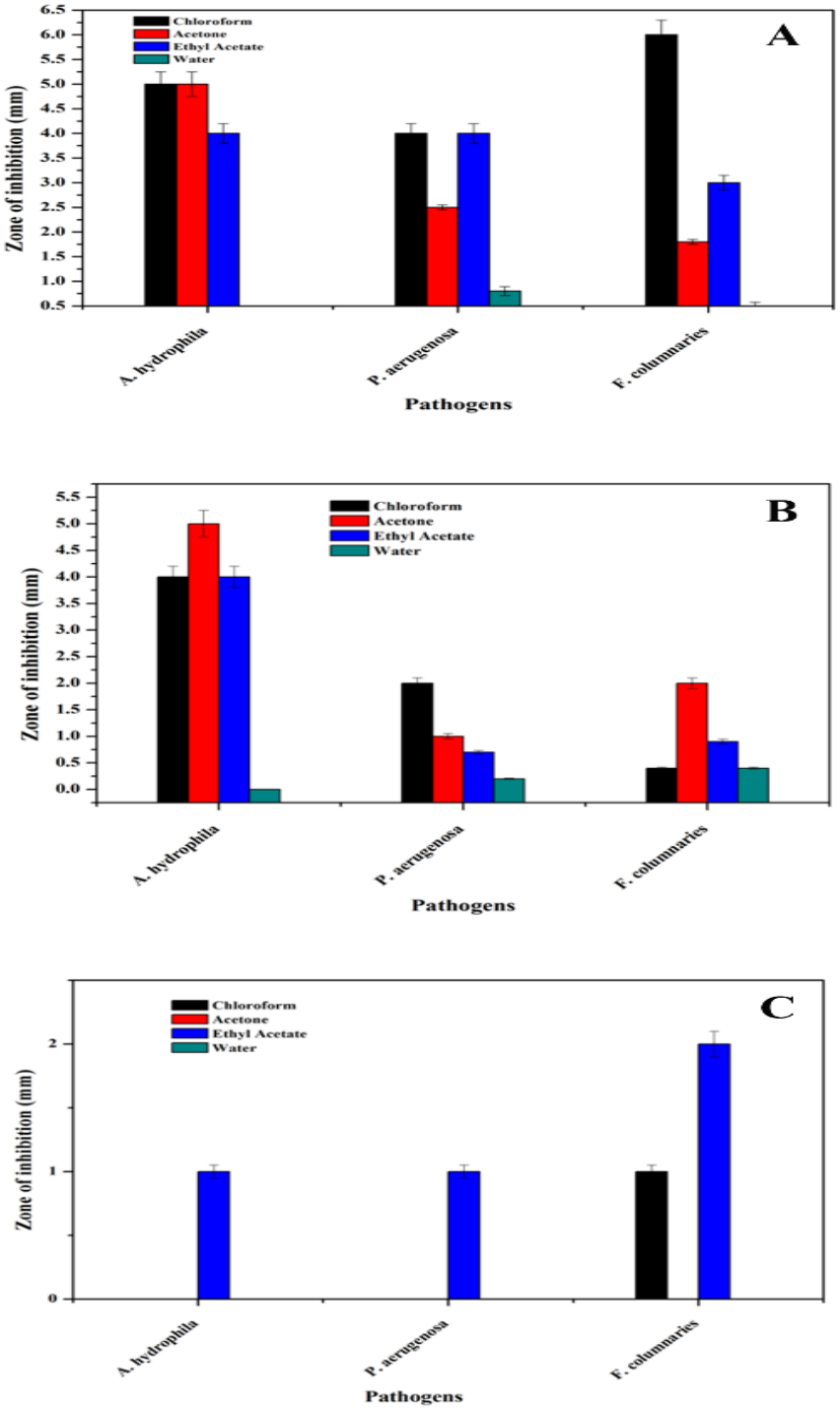

Figure 7: Bar graph showed the antibacterial activity of a different solvent extract of *A. indica* (A), *M. oleifera* (B), *C. dactylon* (C) against *A. hydrophila*, *P. aeruginosa*, *F. columnaris.*

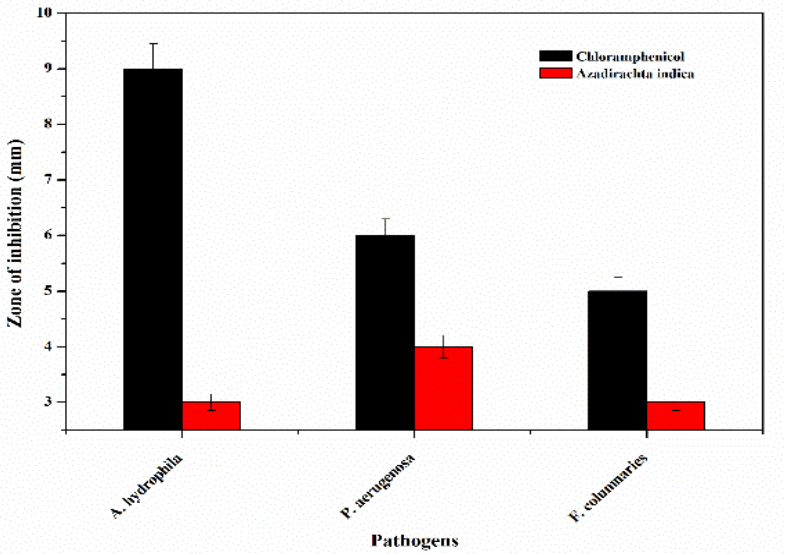

Figure 8: Bar graph shows the biocidal activity of *Azadirachta indica* plant extract and Chloramphenicol.

affected by bacterial. The main part of this study is to analyze the fin regeneration in a normal condition and bacterial infection. Similarly, traditional plant extracts and antibiotics are used for the biocidal activity of infected pathogens. Caudal fin regeneration starts with typically, during the bacterial infection regeneration was begun with a postponement. Chloramphenicol cured the infection in short period and the caudal fin regeneration process regained, and plant extract of *A. indica* take more time to cure the fish disease. *A. indica* indicated the most extreme zone of hindrance against from *F. columnaris, A. hydrophila* and *P. aeruginosa*. By administrating the plant concentrate to the contaminated fishes, it is conceivable to keep the spreading of the malady and furthermore, the sickness can be cured. Our outcomes recommend that nearness of the microscopic organisms in the tainted cells may prompt to the hindrance of recovery in the cutoff district and chloroform concentrate of *Azadirachta indica* could be utilized for the advancement of new sorts of antibacterial specialists and may hence be utilized as remedial in caudal fin recovery of zebra fish.

CONFLICTS OF INTEREST

There are none declared conflicts of interest by authors

REFERENCES

1. Carlson, Bruce M., ed. Principles of regenerative biology. Academic Press, 2011.
2. Mills, Stephanie. In service of the wild: restoring and reinhabiting damaged land. Beacon Press, 1995.
3. Singh SP, Holdway JE, Poss KD. Regeneration of amputated zebrafish fin rays from de novo osteoblasts. Developmental cell 2012; 22(4): 879-86.
4. Ceci ML, Mardones-Krsulovic C, Sánchez M, Valdivia LE, Allende ML. Axon-Schwann cell interactions during peripheral nerve regeneration in zebrafish larvae. Neural development 2014; 9(1): 22.
5. Major RJ, Poss KD. Zebrafish heart regeneration as a model for cardiac tissue repair. Drug Discovery Today: Disease Models 2008; 4(4): 219-25.
6. Poss KD. Advances in understanding tissue regenerative capacity and mechanisms in animals. Nature Reviews Genetics 2010; 11(10): 710-22.
7. Jazwinska A, Rushlow C, Roth S. The role of brinker in mediating the graded response to Dpp in early Drosophila embryos. Development 1999; 126(15): 3323-34.
8. Katogi R, Nakatani Y, Shin-i T, Kohara Y, Inohaya K, Kudo A. Large-scale analysis of the genes involved in fin regeneration and blastema formation in the medaka, Oryzias latipes. Mechanisms of development 2004;121(7): 861-72.
9. Jopling C, Suñé G, Faucherre A, Fabregat C, Belmonte JC. Hypoxia induces myocardial regeneration in zebrafish. Circulation 2012: CIRCULATIONAHA-112.
10. Bayliss PE, Bellavance KL, Whitehead GG, Abrams JM, Aegerter S, Robbins HS, Cowan DB, Keating MT, O'Reilly T, Wood JM, Roberts TM. Chemical modulation of receptor signaling inhibits regenerative angiogenesis in adult zebrafish. Nature chemical biology 2006; 2(5): 265-73.
11. Pandey G. Some medicinal plants to treat fish ectoparasitic infections. International Journal of Pharmaceutical and Research Sciences 2013; 2(2): 532-8.
12. Sousa S, Afonso N, Bensimon-Brito A, Fonseca M, Simões M, Leon J, Roehl H, Cancela ML, Jacinto A. Differentiated skeletal cells contribute to blastema formation during zebrafish fin regeneration. Development 2011; 138(18): 3897-905.
13. Fan LW, Kaizaki A, Tien LT, Pang Y, Tanaka S, Numazawa S, Bhatt AJ, Cai Z. Celecoxib attenuates systemic lipopolysaccharide-induced brain inflammation and white matter injury in the neonatal rats. Neuroscience 2013; 240: 27-38.

14. Sukanya SL, Sudisha J, Hariprasad P, Niranjana SR, Prakash HS, Fathima SK. Antimicrobial activity of leaf extracts of Indian medicinal plants against clinical and phytopathogenic bacteria. African journal of biotechnology 2009; 8(23).
15. Wikler MA. Performance standards for antimicrobial susceptibility testing: Sixteenth informational supplement. Clinical and Laboratory Standards Institute; 2006.
16. Stoick-Cooper CL, Weidinger G, Riehle KJ, Hubbert C, Major MB, Fausto N, Moon RT. Distinct Wnt signaling pathways have opposing roles in appendage regeneration. Development 2007; 134(3): 479-89.
17. https://source.wustl.edu/2011/05/zebrafish-regrow-fins-using-multiple-cell-types-not-identical-stem-cells/dated on 18.03.2017.
18. Mäurer M, Loserth S, Kolb-Mäurer A, Ponath A, Wiese S, Kruse N, Rieckmann P. A polymorphism in the human cytotoxic T-lymphocyte antigen 4 (CTLA4) gene (exon 1+ 49) alters T-cell activation. Immunogenetics 2002; 54(1): 1-8.
19. Wang Z, Hang S, Purdy AE, Watnick PI. Mutations in the IMD pathway and mustard counter Vibrio cholerae suppression of intestinal stem cell division in Drosophila. MBio. 2013; 4(3): e00337-13.
20. Schadich E, Cole AL. Pathogenicity of Aeromonas hydrophila, Klebsiella pneumoniae, and Proteus mirabilis to brown tree frogs (Litoria ewingii). Comparative medicine 2010; 60(2): 114-7.
21. Phennicie RT, Sullivan MJ, Singer JT, Yoder JA, Kim CH. Specific resistance to Pseudomonas aeruginosa infection in zebrafish is mediated by the cystic fibrosis transmembrane conductance regulator. Infection and immunity 2010; 78(11): 4542-50.
22. Bernardet JF. France and comparison with bacterial strains from other origins. Diseases of Aquatic Organisms 1989; 6: 37-44