Use of *Rhizophora* sp. leaf extract for healing motile *Aeromonas septicemia* disease on catfish (*Pangasius hypophthalmus*)

H Syawal*, R Karnila, U M Tang, I Effendi

Faculty of Fisheries and Marine, University of Riau, Kampus Bina Widya, Jalan HR. Soebrantas KM. 12,5, Simpang Baru, Tampan, Riau, 28292

*Email: henni.syawal@lecturer.unri.ac.id*

**Abstract.** Catfish (*Pangasius hypophthalmus*) is a commercially grown aquatic animal, and *A. hydrophila* has been described to often cause disease to the Motil Aeromonas Septicemia (MAS) specie. In addition, *Rhizophora* sp. is a plant reported to contain steroidal compounds, saponins, flavonoids, and tannins, and flavonoid content is widely found in mangrove plants, especially in its fruit, roots, leaves, and outer bark. This study, therefore, aims to examine the uses of *Rhizophora* sp. leaves extract in the treatment of catfish infected by *A. hydrophila*, conducted using a completely randomized design (RAL) of one factor (dose of treatment with extract), consisting of four levels; T1 (1300 ppm), T2 (1600 ppm), T3 (1900 ppm), and CT (0 ppm) serving as a control. Moreover, the leaves were sliced, air dried in the room for 10 days, milled, and then macerated with 95% ethanol for 24 hours. This dough was filtered and the residue was further macerated 3 more times, and the resulting filtrate was blended and evaporated to the point at which the gel-shaped extract was obtained. Furthermore, the catfish were injected intramuscularly with 0.1 mL / fish suspension of *A. hydrophila* bacteria, and the treatment was conducted twice (18 and 42 hours post injection) by soaking in a solution of The leaves for 5 minutes. Parameter measurements (total erythrocytes, hemoglobin levels, hematocrit) values, and graduation of fish life were evaluated at day 0, 3 and 14, post administration and the results showed a decreased in the assessed parameters of the infected fish, when compared to normal ones, which was further elevated close to that shown by the normal, on the 14th day. Post-healing treatment survival rate is observed to be influenced by the concentration administered, and the results were as follows; T1 (33.30 %), T2 (84.60 %), T3 (66.60 %) and CT (0 %).

1. **Introduction**

*Rhizophora* is a genus of tropical mangrove trees, collectively referred to as true mangroves, broadly spread through the Indonesian coastal area. Meanwhile, they are characterized richly of steroid compounds, saponins, tannins, and flavonoids, widely found in their fruits, roots, leaves, and outer bark [1-4].

Furthermore, they are a group of plants rich in secondary metabolites, encompassing natural alkaloids, flavonoids, phenolics, steroids, and terpenoids, proven to possess antioxidant, antibacterial, anti-tumor,
and anti-viral properties [5, 6] as well as anti-fungi [7]. Furthermore, Syawal and Karnila [2] reported its leaves to contain high flavonoid content in comparison with other parts of the plant.

In addition, its benefits to humans include protecting the cell structure, increasing the effectiveness of absorption, and use of vitamin C in the body, its anti-inflammatory, antiviral, as an antibiotic properties, and also the assistance towards improving the immune system [8-10]. Furthermore, plant products have also been adopted as cheap and the potential source of medicinal ingredients [11].

Catfish (Pangasius hypophthalmus) is grown commercially in most countries of South East Asia, with the consistent exploration of their rapid development in ponds, tanks or cages. Moreover, it tastes good, and the selling price is relatively high, hence, fish farming is very popular in the society. in addition, the intensity of its cultivation as a business has increased dramatically, and the decreasing quality of water in the environment has recently lead to the incidence of catastrophic diseases.

The bacterium, Aeromonas hydrophila is an opportunistic pathogen to some fresh water fish, including catfish, which occurs mainly in poor condition [12]. However, the disease caused by its pathogenicity is termed Motil Aeromonas Septicemia (MAS), which spreads very fast, and confers a very high mortality in a relatively short period of time [13]. Furthermore, clinical symptoms of fish infected with this microorganism includes sluggish movements, leading to dormancy at the bottom of the aquarium, wound/ulcers in the infected area, bleeding at the base of the caudal and dorsal fin, as well as the appearance of visibly bloated and swollen lower abdomen. In addition, before death, the affected fish rises to the surface of the water with an unstable swimming pattern [14]. This study is, therefore, aimed at examining the use of Rhizopora sp. leaf extract in the treatment of catfish infected by A. hydrophila.

2. Material and Methods
Mangrove leaves of Rizhopora sp. were collected from the conservation area of Dumai City, Riau Province, while the Catfish (Pangasius hypophthalmus), with sizes ranging from 8-10 cm in length, were obtained from a farm in Pekanbaru City. Furthermore, isolates of Aeromonas hydrophila were provided by Fish and Parasite Disease Laboratory, Faculty of Fisheries and Marine Sciences, University of Riau.

This study involved an experimental method, using a completely randomized design (CRD) of one factor (dose of healing treatment with Rizhopora sp. leaf extract). This consisted of four levels, including T1 (1.300 ppm), T2 (1.600 ppm), T3 (1.900 ppm) and CT (0.00 ppm), which served as a control), all of which were conducted in three replications. Furthermore, the determination of doses was based on the results of the toxicity test (LD50) of the leaf extract, against tilapia [2].

Rizhopora sp. leaves were sliced into small sizes, and air dried in the room for 10 days. This was milled, using a blender, and macerated with 95% ethanol (1: 5) for 24 hours. Furthermore, the mixture was filtered and the residue was, therefore, macerated 3 times. This filtrate obtained was combined and evaporated using a rotary evaporator at a temperature of 37°C to the point where a gel-shaped extract was formed.

Catfish were infected by intramuscular injection at 0.1 mL/fish suspension dose of A. hydrophila 10⁵ CFU / mL bacterial suspension. Therefore, the infected fish were placed in an aquarium, measuring 40 x 30 x 30 cm, with a density of 1 fish / 2.5 l of water, and the curative treatment was conducted twice. Moreover, the first occurred 18 hours after infection, while the second was on the 42nd hour, through immersion in a solution of the leaves extract for 5 minutes.

Parameter measurements were taken at the beginning, after infection, and on the 14th day post-remedial treatment. These include total erythrocytes, hemoglobin levels, hematocrit values, and survival rate of fish at the termination of study.
3. Results and Discussions

The results of this study indicated that the immersion treatment in a solution of Rhizopora sp. leaf extract caused resolving effects on infected catfish. This was noted from the decrease in the total of erythrocytes, hemoglobin and hematocrit levels, in comparison with the normal hematological value. Furthermore, this value increased again, approaching the normal expectation on day 14, as details were presented in Table 1.

Table 1. Total of erythrocytes, hemoglobin levels and hematocrit levels of catfish infected with A. hydrophila

| Parameter                        | CT (0)          | T1 (1.300)      | T2 (1.600)      | T3 (1.900)      |
|----------------------------------|-----------------|-----------------|-----------------|-----------------|
| Total Eritrocytes x 10⁴ cel.mm⁻³ | 141.00 ±1.00    | 141.00 ±1.00    | 141.00 ±1.00    | 141.00 ±1.00    |
| Day 0                            |                 |                 |                 |                 |
| Day 3                            | 111.00±2.65     | 110.67±8.08     | 111.67±5.69     | 111.33±3.51     |
| Day 14                           | 0.00 ± 0.00     | 105.67±21.57    | 346.00±22.60    | 274.67±22.8     |
| Haemoglobin level gdL⁻¹          |                 |                 |                 |                 |
| Day 0                            | 7.87 ± 0.30     | 7.87 ± 0.30     | 7.87 ± 0.30     | 7.87±0.30       |
| Day 3                            | 6.7±0.47        | 6.8±0.6         | 6.9±0.20        | 6.9±0.50        |
| Day 14                           | 0.00 ± 0.00     | 7.00 ± 0.50     | 9.30 ± 0.46     | 8.60± 0.30      |
| Kadar Haematocrit %              |                 |                 |                 |                 |
| Day 0                            | 31.00 ± 1.00    | 31.00 ± 1.00    | 31.00 ± 1.00    | 31.00 ± 1.00    |
| Day 3                            | 26.60 ± 1.73    | 26.33 ± 1.52    | 26.67 ± 1.52    | 26.33 ± 1.52    |
| Day 14                           | 00.00 ± 0.00    | 23.00 ± 1.00    | 30.67 ± 1.53    | 28.67 ± 2.08    |

Based on the data in Table 1, it is noted that the parameters evaluated, encompassing total erythrocytes, hemoglobin levels, and hematocrit values of post-infection fish, decreased comparatively with the normal ones. This occurred due to the high virulence of A. hydrophila, which possesses the aerolysin and hemolysin toxins, capable of lysing red blood cells [15, 16]. Furthermore, Majum et al [17] and Nahar et al [18] reported the presence of plasmids to play an important role in the staining, growth, and virulence of A. hydrophila, as well as the pathogenesis of ulcerative disease syndrome (USD). The decrease in total erythrocytes has an impact on the reduction in hemoglobin levels and hematocrit values, which occurs because they are directly proportional to each other.

These values increased after the remedial treatment with mangrove leaves extract. Moreover, this occurrence was thought to be due to the presence of flavonoids, as Syawal and Karnila [2] reported the presence of 4.38 ppm (0.9113% w/w). Chifdhiyah [19] also stated its activity as an antioxidant, based on the active polyphenols and immunomodulators content, capable of increasing phagocytic cell activity. Hence, this curative role takes place through phagocytosis against the pathogen cells.

Furthermore, other benefits of flavonoids also includes the protection of cell structures, increase in the effectiveness of absorption and use of vitamin C in the body, as well as its anti-inflammatory, antiviral, as antibiotics capacity, which helps to improve the immune system [9, 10, 19]. In addition, the destruction of phagocytic microbial particles involves the release of enzymes into phagosomes and the subsequent production of ROI (reactive oxygen intermediate), therefore, called respiratory burst [20].

Post-healing treatment catfish survival rate is seen to be influenced by the concentration of Rhizopora sp extract, as all four remedial treatment levels including T1 (33.30 %), T2 (84.60 %), T3 (66.60 %) and CT (0 %), and the results presented in Table 2. Moreover, the highest percentage was achieved in T2 (1,600 ppm) experimental unit, which lead to 84.60% survival rate, indicating the capability of the extract content, encompassing flavonoids and antioxidants to restore the condition of fish infected with A.
hydrophila. This is indicated by the haematological values returning to normal, e.g., erythrocytes (1.6 - 2.4x106 cells/ mm-3), hemoglobin (4-14.6 gDl-1), and hematocrit (22.7-39.3 %) [21].

Table 2. Survival rate (%) of infected catfish before and after healing treatment.

| Treatment | Number of Infected Fish | Post-healing treatment I | Post-healing treatment II | Post-healing treatment III | Final survival rate |
|-----------|-------------------------|--------------------------|---------------------------|----------------------------|---------------------|
| CT        | 30                      | 13.33                    | 0                         | 0                          | 0                   |
| T1        | 30                      | 60.00                    | 20.00                     | 6.00                       | 33.30               |
| T2        | 30                      | 60.00                    | 43.33                     | 36.66                      | 84.60               |
| T3        | 30                      | 56.67                    | 40.00                     | 26.66                      | 66.60               |

The increase in fish body defense was due to the phytochemical content of Rhizophora sp. leaf extract which include tannins, terpenoids, saponins, and flavonoids [22]. In addition, Tannins functioned by coagulating bacterial cell cytoplasm, which result in bacterial death [23]. Triterpenoid or Steroidal compounds possessed the capacity to inhibit bacterial growth through the restraining mechanisms on protein synthesis as it accumulates, therefore, causing changes in the components of bacterial cells [24]. Hence, all components of this secondary metabolite possess the capacity to increase post infected fish immunity, in order to enhance their ability to fight extracellular products of A. hydrophila, characterized by the high survival rate reported.

4. Conclusion

The procedure of soaking in a solution of Rizophora sp extract resulted a remedial treatment effect on catfish infected by A. hydrophila. Moreover, the results showed that total erythrocytes, hemoglobin, and hematocrit values decreased in the infected fish, when compared to normal parameters. This number increased again close to normative values on day 14 post-remedial treatment. Furthermore, survival rate was seen to be influenced by the concentration of Rizophora sp extract, as follows T1 (33.30%), T2 (84.60%), T3 (66.60%) and CT (0%). Therefore, further research on the effectiveness of this therapy on a field scale is recommended.

Acknowledgement

The authors express their gratitude to the Institute of Research and Community Service, University of Riau, for funding this research, and also the Dean and staff of the Faculty of fisheries and Marine, for the assistance provided.

References

[1] Wibowo C, Kusmana C, Suryani A, Hartati Y and Oktadiyani P 2009 Prosiding Hasil-Hasil Penelitian IPB 2009 158-166
[2] Syawal H and Karnila R 2016 Kumpulan Abstrak International Conference on Aquaculture Biotechnology
[3] Ridlo A, Pramesti R, Supriyantini E and Soenardjo N 2017 Buletin Oceanografi Marina 6(2) 110-116
[4] Soonthornchareonnon N, Wiwat C and Chuakul W 2012 Mahidol University J of Pharmaceutical Science 39 9-18
[5] Sharief N M D and Rao U M V 2014 *Journal of Chemical and Pharmaceutical Research* **10** 252-256
[6] Rastegar S and Gozari M 2017 *Journal of Paramedical Sciences (JPS)* **8**(1) 1-6
[7] Rohyami Y 2008 *Jurnal Logika* **5**(1) 1-8
[8] Haris M 2011 Skripsi Fakultas Farmasi, Universitas Andalas, Padang
[9] Rosnizan R, Maulida S, Eriani K and Suwanto 2017 *Jurnal Bioleuser* **1**(3) 104-115
[10] Saptiani G, Hardi EH, Pebrianto CA, Agustina and Ardhani F 2016 *Nusantara Bioscience* **8**(2) 252-257
[11] Citterio and Biavasco 2015 *Virulence* **6**(5) 417–418
[12] Harikrishnan R, Nisha RM and Balasundaram C 2003 *Aquaculture* **221** 41-50
[13] Jian J and Wu Z 2004 *Fish Shellfish Immunol* **16** 185-191
[14] Rey-Lopez JP, Vicente-Rodriguez G, Biosca M and Moreno LA 2008 *Nutrition, Metabolism and Cardiovascular Diseases* **18**(3) 242-251
[15] Lukistyowati I and Kurniasih 2012 *Jurnal Veteriner* **13**(1) 43-50
[16] Majumdar T, Datta S, Ghosh D, Dutta S, Chakraborty A, Goswami and Smazumder R 2007 *Indian Journal of Biochemistry & Biophysics* **44** 401-406
[17] Nahar S, Rahman MM, Ahmed GU and Farok MAR 2016 *International Journal of Fisheries and Aquatic Studies* **4**(4) 52-60
[18] Chifdhiyah AN 2012 *Journal of Aquaculture Management and Technology* **1**(1) 35-47
[19] Manoppo H 2011 Disertasi Sekolah Pascasarjana IPB, Bogor
[20] Stoskopf MK 1992 *Fish Medicine* Philadelphia WB, Saunders Company & Harcourt Brace Jovanovich, Inc
[21] Syawal H, Karnila R, Ditra A and Kurniawan R 2017 *Jurnal Veteriner* **18**(4) 1-6
[22] Karlina CY, Ibrahim M and Trimulyono G 2013 *Journal UNESA Lentera-Bio* **2**(1) 87–93
[23] Yin G, Ardo L, Thompson KD, Adams A, Jeney Z and Jeney G 2010 *Fish and Shellfish Immunology* **26**(1) 140-146