**Biofunctional Properties of *Pluchea indica* L. Leaf Extract in**

***Litopenaeus vannamei* Culture: Antibacterial and Antioxidant Efficacy with Toxicity Assessment**

**ABSTRACT**

Innovations in utilizing the phytonutrient content of natural ingredients, one which is *P. indica* leaf in whiteleg shrimp farming, has various functional benefits especially as a natural medicine for shrimp health. This study aimed to investigate the antibacterial and antioxidant potential of P. indica leaf extract and to evaluate its toxicity level toward whiteleg shrimp. Antibacterial activity was assessed using the well diffusion method, while antioxidant capacity was measured through the DPPH assay. Toxicity was determined based on the 96-hour LC50 test. Phytochemical screening revealed that the P. indica leaf extract contains bioactive compounds, including alkaloids, saponins, polyphenols (flavonoids, tannins, and phenols), triterpenoids, and glycosides, which contribute to its functional properties. P. indica leaf extract exhibited an LC50 value of 432.51 ppm which has toxic potential for whiteleg shrimp, provides antibacterial activity from a concentration of 1% with a weak category of 1.01 ± 0.43 mm and becomes stronger as the concentration increases with the highest result of the V. parahaemolyticus bacterial inhibition zone of 13.72 ± 1.09 mm at a concentration of 100% and was classified as a strong antioxidant agent (average EC50 value of 82,630±2.76 ppm).

**Keywords**: *antibacterial, antioxidant, P. indica leaf, whiteleg shrimp, V. parahaemolyticus*

**INTRODUCTION**

Aquaculture products play a vital role in ensuring global food security by serving as a significant source of high-quality animal protein. Among crustacean aquaculture commodities, whitelegi shrimp dominates the global market and is projected to grow at an annual rate of 6.72% through 2028, reaching an estimated market value of USD 69.35 billion (Cheney, 2024). This growth is largely attributed to the species’ superior characteristics, including rapid growth rates, efficient feed conversion, and high adaptability to various environmental conditions (Chen *et al.,* 2020). The high intensity of shrimp farming activities to achieve production targets is directly proportional to the increase in feed consumption, thus increasing the burden of organic matter on the bottom of the waters.

Organic matter that increasingly accumulates at the bottom of the water has a significant impact on environmental quality and shrimp health, such as decreased oxygen levels, disease outbreaks such as vibriosis, and decreased productivity (Ariadi *et al.,* 2025). These conditions exacerbate the challenges in aquaculture systems, as stressed shrimp become more susceptible to pathogenic agents, relying solely on non-specific immune responses for defense. Unlike specific immunity, the non-specific immune system of shrimp lacks immunological memory and targeted responses to pathogens, depending instead on physical barriers such as the exoskeleton and mucous layers. These physical defenses are often compromised under stress conditions (Natrah *et al.,* 2025). Therefore, improvements and innovations in handling some aquaculture problems, such as handling pathogen infections and counteracting stress in shrimp using safe methods, need to be considered to replace the role of antibiotics, which have undesirable environmental consequences.

The advancement in the use of environmentally friendly natural ingredients in whiteleg shrimp farming has shown various promising benefits. One of them is the leaf of the *P. indica* plant which has the potential content of phytonutrient compounds that can act as a natural medicine that is beneficial for the health of living organisms (Putra *et al.,* 2025). According to Linayati *et al.,* (2022) *P. indica* leaves have the potential as an antioxidant agent that can help the growth of whiteleg shrimp. Furthermore, studies by Al-ashkar *et al.,* (2023) have demonstrated the antibacterial properties of P. indica leaves against several Gram-positive and Gram-negative bacteria, including *B. subtilis, C. albicans, C. neoformans, E. faecalis, E. coli, S. Aureus*. Currently, there is still a lack of use of *P. indica* leaf as antibacterial and antioxidant agents in whiteleg shrimp so that the purpose of this study examines the potential of *P. indica* leaf as antibacterial and antioxidant agents and the level of toxicity to whiteleg shrimp.

**MATERIALS AND METHODS**

The research was conducted at the Microbiology Laboratory of Pekalongan University for 30 days in July 2024. The test organisms used were whitelegi shrimp with an average body weight of approximately ± 4 g, obtained from ponds located near the research site, and *Vibrio parahaemolyticus* used in the study were sourced from the culture collection of the Microbiology Laboratory, Pekalongan University. Shrimp rearing containers using aquarium 40x30x20 cm with a capacity of 30 L and media water using sea water that has been filtered before.

**Extraction Procedure of P. indica Leaf**

*P. indica* leaves were collected from coastal areas around Pekalongan, selected based on criteria including complete leaf morphology and fresh green coloration. Leaves were harvested starting from the first three stem segments from the base. The selected leaves were then thoroughly cleaned and oven-dried to produce simplisia. Simplisia was ground into powder form in order to expand contact with the solvent (Abubakar & Haque, 2020). A total of 100 g of powdered simplisia was weighed and placed into a jar and macerated with 96% ethanol in a ratio of 1:10 (Yunita *et al.,* 2023). The mixture was stirred and tightly sealed, and the process is repeated 3 times every 24 hours to maximize extraction efficiency. After 3 x 24 hours, the mixture was filtered using No. 1 filter paper to obtain the filtrate. The resulting extract was then concentrated using a rotary evaporator to produce a thick extract.

**Phytochemical Studies**

The qualitative phytochemical analysis was conducted following the method of (Indriaty *et al.,* 2023):

Alkaloids: A 1 mL sample of the *P. indica* leaf extract filtrate was mixed with 5 drops of H₂SO₄ and 5 drops of Dragendorff’s reagent. A positive reaction was indicated by the appearance of a yellow to reddish precipitate

Flavonoids: A 5 mL sample of the extract filtrate was treated with 1 mL of concentrated HCl and 0,05 g of magnesium powder. A color change to red or yellow indicated the presence of flavonoids.

Tannins: A 1 mL of the extract was mixed with a 1% gelatin solution containing NaCl. The formation of a white precipitate indicated a positive result for tannins.

Phenols: A 1 mL sample of the extract was placed in a test tube and treated with a few drops of FeCl₃ solution. A blackish green color indicated the presence of phenolic compounds.

Saponins: The extract was diluted with distilled water at a 1:1 ratio and shaken for 30 seconds, followed by the addition of 2N HCl. The formation of stable foam indicated the presence of saponins.

Steroids and Triterpenoids: A 0,5 g sample of the concentrated extract was mixed with 2 drops of concentrated H₂SO₄ and 10 drops of acetic acid (CH₃COOH). A red or purple color indicated the presence of triterpenoids, while a blue or green color indicated the presence of steroids.

Glycosides: A 1 mL sample of the filtrate was treated with Molisch reagent (α-naphtol solution in ethanol) and a few drops of concentrated H₂SO₄. The appearance of a reddish-purple ring at the interface indicated the presence of glycosides.

**Antibacterial Activity Test of P. indica Leaf Extract**

The antibacterial activity of P. indica leaf extract was evaluated using the well diffusion method (Wahyuni *et al.,* 2024). Mueller-Hinton Agar (MHA) was used as the culture medium for bacterial isolation and growth. Prior totesting, the turbidity of the *V. parahaemolyticus* bacterial suspension was adjusted by comparing it to the 0,5 McFarland standard, which corresponds to a bacterial concentration of approximately 10⁸ CFU/mL and served as the stock solution. This study used a *V. parahaemolyticus* bacterial colony density of 106 CFU/mL resulting from a multistage serial dilution of the stock suspension and spread over the surface of the MHA media evenly using a sterile cotton swab (Coelho *et al.,* 2021).

The stock solution of *P. indica* leaf extract was prepared by weighing 10 g of extract and diluting it with 96% ethanol to obtain a final volume of 10 mL. A 6 mm diameter well was then prepared and filled with 50 µL of the P. indica leaf extract solution using a micropipette, at concentrations in tenfold serial dilutions (Azhari *et al.,* 2018), namely: 100%, 10%, 1%, 0,1%, 0,01%, 0,001%, and 0,0001%. Ethanol (the extract solvent) was used as the negative control, and Oxytetracycline was used as the positive control. The procedure was conducted under a laminary airflow cabinet. The solution was applied using a micropipette and carefully dispensed into each well on the MHA medium, with each concentration tested in five replicates.

The media then incubated at 37oC for 24 hours. After incubation, the clear zones formed around the wells were observed and their diameters measured. The inhibition zones formed around the wells were classified according to the criteria described by Winastri *et al.,* (2020), as presented in Table 1.

Table 1. Inhibition Zone Response Criteria

|  |  |
| --- | --- |
| Inhibition Zone Diameter | Response Category |
| ≥ 20 mm | Very Strong |
| 10 - 20 mm | Strong |
| 5 - 10 mm | Moderate |
| < 5 mm | Weak |

**Antioxidant Activity Test of *P. indica* Leaf Extract**

The antioxidant activity test of *P. indica* leaf extract was carried out using the DPPH (2,2-diphenyl-1-picrylhydrazyl) method (Baliyan *et al.,* 2022). A total of 3 mL of each concentration of the P. indica leaf extract and a blank were placed into separate test tubes and mixed with 3 mL of 0,05 mM (Mr DPPH 394.32 g/mol) in a 1:1 ratio. The mixed solution was incubated in a dark room for 30 minutes. Absorbance was measured using a UV-Vis spectrophotometer at a maximum wavelength of 517 nm. The percentage of inhibition was calculated using the formula (Kumara *et al.,* 2018).

The percentage of inhibition results were used to generate a linear regression equation in the form of “Y = bx + a,” which was then used to determine the EC50 value, (the concentration required to inhibit 50% of free radicals).

**Toxicity Test of *P. indica* Leaf Extract**

The toxicity test of P. indica leaf extract was conducted using 10 whiteleg shrimp with an average body weight of 4 g per individual. The shrimp were maintained in container boxes filled with seawater at a density of 1 shrimp/3 L. Each container was treated with different concentrations of P. indica leaf extract: 0, 1, 10, 100, and 1000 ppm, for preliminary testing (Fardiaz *et al.,* 2023). For 2 x 24 hours, the mortality rate of whiteleg shrimp was observed to produce lower (n) and upper (N) threshold values. The resulting threshold value is used as the basis for the concentration of *P. indica* leaf extract for lethal tests using Quantal Responses (Abu *et al.,* 2022):

Description:

N = Threshold concentration

n = Lower threshold concentration

a = Smallest concentration in the concentration series used

b,c,d = Concentration b,c and d in the concentration series used

x = Concentration x in the concentration series used

k = Number of concentration intervals tested

After determining the concentration of *P. indica* leaf extract from the preliminary calculations, the lethal toxicity test was continued following the same procedures. However, in this stage, the mortality rate was observed over a period of 4 x 24 hours to determine the LC50 value (the concentration causing 50% mortality).

**Data Analysis**

The bacterial inhibition data were analyzed using ANOVA after confirming normality and homogeneity assumptions through the Liliefors test and Bartlett test, respectively. Followed by Turkey test to determine the difference between concentrations of bacterial inhibition produced. Meanwhile, LC50 value was determined using probit analysis, and the EC50 value was calculated from a linear regression equation with the assistance of Microsoft Excel software.

**RESULTS**

**Phytochemistry of P. indica Leaf Extract**

The results of the identification of phytonutrient compounds present in P. indica leaf extract are summarized in Table 2.

Table 2. Identified Phytonutrient Compounds

|  |  |  |
| --- | --- | --- |
| **Identified Compounds** | **Result** | **Reaction Produced** |
| Alkaloids | Positive (+) | Produced a reddish-yellow color |
| Flavonoids | Positive (+) | Produced a yellow color |
| Tannins | Positive (+) | Produced a white precipitate |
| Phenols | Positive (+) | Produced a green color |
| Saponins | Positive (+) | Produced stable foam |
| Triterpenoids | Positive (+) | Produced a purple color |
| Steroids | Negative (-) | No blue or green color formed |
| Glycosides | Positive (+) | Formed a reddish-purple ring |

**Antibacterial Activity of *V.parahaemolyticus* Inhibited by *P. indica* Leaf Extract**

The results showed that bacterial inhibition of *V. parahaemolyticus* by P. indica leaf extract began to appear at a concentration of 1%, which was still categorized as weak. Increasing the concentration of P. indica leaf extract resulted in stronger antibacterial activity. These findings are presented in Table 3.

Table 3. The Inhibiton Zone of *P. indica* Leaf Extract

|  |  |  |
| --- | --- | --- |
| **Treatments** | **Average ± SD** | **Interpretation** |
| Oxytetracyclin (positive control) | 16.65 ± 1.58e | Strong |
| 100% | 13.72 ± 1.09d | Strong |
| 10% | 8.53 ± 0.58c | Moderate |
| 1% | 1.01 ± 0.43b | Weak |
| 0.1% | 0.00 ± 0.00a | - |
| 0.01% | 0.00 ± 0.00a | - |
| 0.001% | 0.00 ± 0.00a | - |
| 0.0001% | 0.00 ± 0.00a | - |
| Ethanol (negative control) | 0.00 ± 0.00 | - |

Note: different notation indicate significant differences among treatments (*p<0.05)*

**Antioxidant Activity of P. indica Leaf Extract**

The results of the linear regression analysis of the % inhibition, based on the sample absorbance readings, indicate that P. indica leaf extract exhibits a strong antioxidant activity. These results are summarized in Table 4.

Table 4. EC50 of *P. indica* Leaf Extract

|  |  |  |  |
| --- | --- | --- | --- |
| **Replication** | **Linier Regression Equation** | **EC50 Value (ppm)** | **Average EC50 Value (ppm)** |
| I | Y = 0,1225X +39,751 | 83,665 | 82,630±2.76 |
| II | Y = - 0,3562X +78,583 | 80,244 |
| III | Y = 0,5962X +2,276 | 80,047 |
| IV | Y = - 0,0247X +52,143 | 86,761 |
| V | Y = 0,1935X +34,049 | 82,434 |

**Toxicity of *P. indica* Leaf Extract on Whitelegi Shrimp**

The results of the preliminary test on the mortality rate of whiteleg shrimp over 2 x 24 hours are presented in Table 5.

Table 5. Mortality of Whiteleg Shrimp in Preliminary Test

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Concentration of *P. indica* Leaf Extract** | **Initial Spread (head)** | **Mortality Observations** | **Total Number of Deaths (head)** | **Mortality Rate (%)** |
| **24 hours** | **48 hours** |
| 0 ppm (Control) | 10 | 0 | 0 | 0 | 0% |
| 1 ppm | 10 | 0 | 0 | 0 | 0% |
| 10 ppm | 10 | 0 | 0 | 0 | 0% |
| 100 ppm | 10 | 0 | 0 | 0 | 0% |
| 1000 ppm | 10 | 2 | 4 | 6 | 60% |

Based on Table 5, the lower threshold value (n) was determined to be 100 ppm, and the upper threshold value (N) was 1000 ppm. These values were used as reference points for the LC50-96 hours lethal toxicity test. Logarithmic calculations using the Quantal Response method, the results of preliminary tests produce P. indica leaf extract concentrations used for lethal toxicity tests that can be observed in Table 6 and the average mortality rate of whiteleg shrimp from 5 repetitions of lethal toxicity tests (LC50-96 hours) of P. indica leaf extract can be observed in Table 7.

Table 6. Post-Calculation Concentration Changes

|  |  |
| --- | --- |
| **Initial Concentration** | **Final Concentration** |
| 0 ppm (control) | 0 ppm (control) |
| 1 ppm | 177.83 ppm |
| 10 ppm | 316.23 ppm |
| 100 ppm | 562.33 ppm |
| 1000 ppm | 1000 ppm |

Table 7. Mortality of Whiteleg Shrimps LC50-96 hours

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Concentration of *P. indica* Leaf Extract** | **Initial Spread (head)** | **Average Mortality Observations** | **Average Total Number of Deaths (head)** | **Mortality Rate (%)** |
| **24** **hours** | **48 hours** | **72 hours** | **96 hours** |
| 0 ppm  | 10 | 0 | 0 | 0 | 0 | 0 | 0% |
| 177.83 ppm | 10 | 0 | 0 | 0.2 | 0.6 | 0.8 | 8% |
| 316.23 ppm | 10 | 0 | 0.6 | 1.2 | 1.8 | 3.6 | 36% |
| 562.33 ppm | 10 | 0.6 | 1 | 2 | 2.2 | 5.8 | 58% |
| 1000 ppm | 10 | 2 | 3 | 3 | 0.8 | 9.2 | 92% |

Based on the results in the table 7, the concentration of *P. indica* leaf extract that caused approximately 50% mortality in whiteleg shrimp was 316.23 ppm. Furthermore, the nearest concentration causing greater than 50% mortality was 562.33 ppm. Therefore, the LC50 value for *P. indica* leaf extract lies between 316.23 and 562.33 ppm. Probit analysis produced a regression equation of Y = 3608X − 45111, as illustrated in Figure 1.

Figure 1. Regression Equation of *P. indica* Leaf Extract Concentration

Based on this regression model, the LC50-96 hours value of *P. indica* leaf extract for whitelegi shrimp was calculated to be 432.51 ppm.

**DISCUSSION**

**Antibacterial Activity**

The result of the inhibition zone test of *P. indica* leaf extract began to form an average inhibition zone against *V. parahaemolyticus* bacteria of 1.01±0.45 mm at a concentration of 1% solution which is still categorized as weak but the higher the concentration of P. indica leaf extract used the higher the inhibition zone formed, namely at a concentration of 10% solution the inhibition zone formed an average of 8.53±0.58 mm with a moderate category and increased the average inhibition zone to a strong category at a concentration of 100% solution with an average of 13.72±1.09 mm. These results are still lower than those reported by Wahyuni et al. (2024), who observed inhibition zones against *E. coli* and *B. subtilis* with average diameters of 31,86 mm and 21,09 mm, respectively, using a 50% P. indica leaf extract concentration. However, the present study demonstrates better antibacterial activity than the findings of Lestari et al. (2020), which reported inhibition zones of only 5,4 mm and 4,8 mm against *E. coli* and *B. subtilis*, respectively, using a maximum concentration of 0,25% *P. indica* leaf extract. The difference in antibacterial ability is due to several factors from the concentration of the ingredients used. According to Kovács et al. (2022), the effectiveness of an antibacterial agent generally increases with concentration, up to a certain threshold. Additionally, differences in species, strains, and bacterial loads affect sensitivity to antibacterial agents (Li *et al.,* 2017). The living environment of antibacterial agents determines the effectiveness of antibacterial agents because different environmental conditions affect the amount and type of active compounds (Lavaee *et al.,* 2021).

The results of secondary metabolite screening of *P. indica* leaf extract revealed the presence of flavonoids, alkaloids, tannins, saponins, phenolics, terpenoids, and glycosides. Alkaloids have broad antibacterial properties with good antibacterial effects against common clinical strains, including drug-resistant bacteria (Yan *et al.,* 2021). In addition, their antibacterial mechanisms include inhibition of nucleic acid synthesis, disruption of peptidoglycan and protein components in bacterial cells, interference with bacterial efflux pump functions, inhibition of metabolic pathways, and modification of bacterial cell membrane permeability. These actions result in structural damage to the bacterial cell wall and membrane, ultimately compromising cell integrity (Zhang *et al.,* 2020). The bacterial efflux pump functions to expel toxic substances from the cell, and its inhibition can enhance antibacterial efficacy (Salas-Orozco *et al.,* 2024).

Flavonoids become antibacterial agents by weakening bacterial pathogenicity and disrupting the cell membrane through protein denaturation, leading to structural changes such as porin alteration and inhibition of energy metabolism (Xie *et al.,* 2015). They also serve as antibiofilm agents, further preventing bacterial growth (Lopes *et al.,* 2017). Additionally, flavonoids can inhibit nucleic acid synthesis by targeting key enzymes such as helicase, dihydrofolate reductase (DHFR), and topoisomerase/gyrase (Górniak *et al.,* 2018).

Some other phenol compounds such as tannins also function in disrupting the rate of bacterial cell protein transport by inhibiting the bacterial fatty acid biosynthesis pathway and tannins also function to weaken virulence factors from bacteria such as toxicity, motility, enzymes, adhesins, and biofilms (Farha *et al.,* 2020). They can also penetrate the bacterial cell wall and membrane, disrupting cellular metabolism and causing membrane damage (Kaczmarek, 2020). Similarly, other phenolic compounds such as preventing enzyme performance, biofilm formation to damage the bacterial cell membrane. Phenolics are able to penetrate to damage bacterial DNA to reach the DNA helix so that they can carry out mechanisms such as replication, recombination to transcription in bacterial DNA and phenolics are able to interact with proteins and cell walls and damage the cytoplasm resulting in inhibition of energy metabolism in bacterial cells (Lubiuc *et al.,* 2023).

Saponins present in *P. indica* leaf extract contribute to damage cell membrane permeability and denature proteins and enzymes of bacterial cells, because saponins have basic properties such as detergents that can erode or reduce the surface tension of bacterial cell walls and result in leakage of proteins and enzymes from the cell and can facilitate the entry of other antibiotic substances into cells through cell wall membranes (Putri *et al.,* 2023).

Terpenoids in *P. indica* leaf extract act as antibacterial agents by interacting with porins in the bacterial cell membrane, forming strong polymers that destroy porins and reduce membrane permeability. This disruption impairs the transport of essential nutrients such as proteins and enzymes, leading to bacterial malnutrition, inhibited growth, or cell death (Aulia *et al.,* 2023). According to Huang et al. (2022), the lipophilic nature of terpenoids allows them to pass through the phospholipid bilayer of bacterial membranes, leading to further structural disruption. Terpenoids can also inhibit essential bacterial enzymes (Mahizan *et al.,* 2019), oxidatively damage nucleic acids and proteins, and inactivate bacterial protein functions (Yang *et al.,* 2021). Additionally, terpenoids are known to inhibit quorum sensing, a bacterial communication system that contributes to antibiotic resistance (Huang *et al.,* 2022).

Glycoside compounds in *P. indica* leaf extract exhibit antibacterial activity by disrupting the bacterial cytoplasm and causing cell lysis. Glycosides can penetrate and degrade the structure of bacterial cell walls by altering membrane permeability, which results in the leakage of cellular components and loss of cell integrity (Tagousop *et al.,* 2018).

**Antioxidant Activity**

The antioxidant activity of *P. indica* leaf extract was measured using the DPPH method (Baliyan *et al.,* 2022). After incubation in a dark room for 30 minutes, absorbance was measured with a UV-Vis spectrophotometer. The results showed that the P. indica leaf extract had an average EC₅₀ value of 82.630 ± 2.76 ppm, which falls into the strong antioxidant category, indicating it is quite effective in scavenging free radicals. According to Fitrasyah *et al.,* (2025), an EC₅₀ value between 50–100 ppm is classified as a strong antioxidant, while values below 50 ppm are categorized as very strong (highly effective). Several previous studies have reported varying EC₅₀ values for *P. indica* leaf extract using the same solvent, including 37.25 ppm (Wanita, 2018), 40.52 ppm (Isromarina *et al.,* 2025), and 94.06 ppm (Sari *et al.,* 2024).

Variations in the EC50 values of P. indica leaf extracts can be attributed to several factors, including environmental conditions, plant genetics, and the extraction process. Phytobiotic compounds produced by plants serve as adaptive responses to environmental stress and as part of their defense mechanisms (Alum, 2024). Therefore, factors such as climate, water availability, sunlight exposure, humidity, and geographical location significantly influence the accumulation of these phytobiotic compounds (Vaou *et al.,* 2022). In addition, genetic factors and the extraction process affect the stability and accumulation of the compounds produced (Tran *et al.,* 2020). Antioxidant activity is particularly associated with the presence of polyphenolic compounds (Baliyan *et al.,* 2022). *P. indica* leaf extract was detected to contain polyphenolic compounds such as flavonoids, tannins, and phenolics. Polyphenols are known for their abundance of electrons, making them highly reactive hydrogen donors, which enables them to effectively scavenge DPPH free radicals (Gulcin & Alwasel, 2023). These compounds typically contain more than one hydroxyl group (-OH), allowing them to bind to multiple aromatic rings and readily interact with DPPH radicals. Free radicals are known to damage essential biomolecules, including proteins and nucleic acids, through oxidative mechanisms (Hayyan *et al.,* 2016). Polyphenolic compounds neutralize free radicals and interrupt the oxidation process by donating a single electron, followed by proton transfer or hydrogen atom donation. This mechanism helps halt the chain reaction of free radical formation, thereby protecting cellular components from oxidative stress (Charlton *et al.,* 2023; Li *et al.,* 2022).

**Toxicity Level of P. indica Leaf Extract Against Whiteleg Shrimp**

The average mortality rate of whiteleg shrimp following the toxicity test of *P. indica* leaf extract reached its highest at 92% mortality at a concentration of 1000 ppm, followed by 58% at 562.33 ppm, 36% at 316.23 ppm, 8% at 177.83 ppm, and 0% in the control group (0 ppm). The results of probit analysis resulted in a regression equation y = 3.608x − 4.5111 (Figure 1). This regression equation results in a 96-hour LC50 value of P. indica leaf extract against whiteleg shrimp is 432.51 ppm. The LC50 value brought to 1000 ppm makes P. indica leaf extract potentially toxic to whiteleg shrimp because according to Setianingsih *et al.,* (2023) the smaller the LC50 value, the higher the toxic level of a compound.

Bioactives derived from plants do have antibacterial, anti-inflammatory, antioxidant, and other therapeutic qualities, but when taken in excessive amounts that the host cannot handle, the bioactive becomes toxic to the host body system. The potential toxicity of flavonoids, in particular, has been reported in association with enzyme interference affecting carcinogenic and mutagenic activity, liver and kidney function, thyroid and reproductive systems, and gastrointestinal health (Tang & Zhang, 2021). At high concentrations, flavonoids may also become harmful due to their ability to bind iron, lipids, and proteins, which poses risks especially for elderly individuals prone to iron deficiency (Birt & Jeffery, 2013).

Alkaloids are secondary metabolic compounds produced by plants that may exhibit toxic effects on both animals and humans, depending on the dosage, duration of exposure, and the specific type of alkaloid involved (Azzeme & Zaman, 2019). The toxicological impact of alkaloids can include disruption of the respiratory and circulatory systems, as well as damage to vital organs such as the liver, nerves, heart, and lungs. In some cases, alkaloids may also act as carcinogenic agents (Shan *et al.,* 2024).

Saponins are another group of bioactive compounds with strong biological activity. These compounds are characterized by a bitter taste and high molecular weight, and they can significantly reduce the surface tension of water, which may interfere with gill function in aquatic organisms (Yasir *et al.,* 2021). At elevated concentrations, saponins have been reported to decrease appetite and reduce egg production. Additionally, they can irritate the digestive and respiratory tracts, damage erythrocyte membranes, and impair blood circulation (Sharma *et al.,* 2023). In shrimp, excessive doses of saponins may inhibit nutrient absorption and protein digestibility, thereby compromising growth and health.

Other compounds present in P. indica leaf extract, such as tannins, are considered anti-nutritional factors that negatively affect protein utilization and digestibility in shrimp (Bolívar-Ramírez *et al.,* 2022). At high concentrations, tannins have been shown to impair growth performance and may be lethal to shrimp (Zhang *et al.,* 2024b). Tannins can also damage the liver, irritate the gastrointestinal tract, and reduce the availability of bioavailable nutrients in the intestine (Sharma *et al.,* 2021). Phenolic compounds, although known for their antioxidant properties, can also exhibit toxic effects. In high doses, phenols may cause skin burns, respiratory tract irritation, liver damage, and hemolysis (Li *et al.,* 2024). Moreover, excessive phenol exposure can lead to reduced reproductive capacity and increased physiological stress, resulting in behavioral changes and, in severe cases, mortality in aquatic organisms, including shrimp.

Terpenoids are known to possess neurotoxic properties that can disrupt the nervous system, leading to hyperactivity and, ultimately, death (Janskowska *et al.,* 2018). These compounds also serve as part of the plant's chemical defense system by producing volatile organic compounds, which can cause physiological and behavioral disturbances in various organisms due to their toxic nature (Câmara *et al.,* 2024). Similarly, glycoside compounds, when present in excessive amounts, have been associated with both neurological and gastrointestinal disturbances (Robert *et al.,* 2015).

**CONCLUSION**

*P. indica* leaf extract contains phytonutrient compounds that exhibit significant bioactivity. It demonstrated strong antibacterial effects against *V.parahaemolyticus*, with a mean inhibition zone of 13.72 ± 1.09 mm at 100% concentration. The extract also showed strong antioxidant activity, with an average EC50 value of 82,630 ± 2,76 ppm based on the DPPH assay. However, the extract exhibited moderate toxicity to whitelegi shrimp, with an LC50 value of 432.51 ppm, indicating the importance of dosage regulation in potential aquaculture applications.

**Disclaimer (Artificial intelligence)**

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

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