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Impact of Addition of Purple Miana Leaf Extract (*Coleus scutellarioides* L. Benth) to Feed on Total Hemocytes and Phagocytic Activity of the Black Tiger Prawns (*Penaeus monodon*)

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ABSTRACT

Miana leaves are known to possess bacterial inhibitory properties comparable to those of antibiotics and can be used to treat vibriosis in shrimp. However, the bioactive compounds in miana leaves and their potential as immunostimulants in feed, particularly their effects on total hemocytes and the phagocytic activity of the tiger prawns, have not yet been fully explored. The experiment used miana leaf extract in feed at concentrations of 0, 10, 20, and 40g/ kg. Bioactive compounds were analyzed using Gas Chromatography-Mass Spectrometry (GC-MS), and statistical analysis of total hemocytes, phagocytic activity, and the tiger prawn survival was performed using the SPSS program. The analysis identified 100 chemical compounds in the ethanol fraction of miana leaf extract. Among these, the three compounds with the highest peak areas were: carbamic acid, methyl ester (CAS methyl carbamate) at 21.13%; 4(5H)-Thiazolone, 2-amino- (CAS *pseudothiohydantoin*) at 16.16%; and Cyclotrisiloxane, hexamethyl- (CAS 1,1,3,3,5,5-hexamethyl-cyclohexasiloxane) at 20.50%. The experimental results showed that the miana leaf extract significantly affected phagocytic activity and survival but did not influence the total hemocytes of the tiger prawns. The highest values for phagocytic activity, survival, and total hemocytes were observed in the 40g/ kg treatment, with values of 76%, 6.25 x 10^5 CFU/mL, and 86.67%, respectively. In conclusion, miana leaf extract contains active antibacterial, antiviral, and anti-inflammatory compounds, and it enhances total hemocytes, phagocytic activity, and the survival of the tiger prawns.

INTRODUCTION

Shrimp cultivation production in Indonesia experiences export fluctuations from year to year. The discourse on cultivation, high production, and the risk of disease attacks and crop failure, is a discussion that is always interesting to develop. The tiger prawns have long been a commodity that is in great demand by domestic and foreign consumers. The tiger prawns are a favorite and a promising export commodity that is promising for cultivation. Farmers try to cultivate the tiger prawns intensively and supra-intensively with the aim of getting high production. This effort was made to meet the fairly high

market demand for tiger prawns. However, in the last few decades, the tiger prawns in ponds have experienced various cultivation problems which have caused quite high losses.

Based on the results of identifying problems with the tiger prawn cultivation, there are at least four factors that cause the tiger prawn grow-out ponds to fail to produce, namely low seed quality, viral and bacterial infections, the carrying capacity of the land which tends to decrease, the environment where cultivation is contaminated, and the extreme environmental fluctuations in the pond due to eutrification **(Supito *et al.*, 2017)**. Infectious diseases are often found as a cause of death in shrimp in ponds and hatcheries **(Hidayani *et al*., 2015; Prabowo *et al*., 2015)**, and often even cause crop failure. Infectious diseases are generally caused by attacks of bacteria and viruses. Prevention of disease infections in shrimp farming is important due to the increasing pollution which poses a risk of damage to the farming environment. Prevention efforts are carried out by increasing the shrimp immune system through the use of immunostimulants in feed.

Natural bioactive ingredients have been widely studied for their use in increasing immunity, or as natural medicines for infectious diseases in shrimp. Natural ingredients that contain active compounds that have the potential to act as immunostimulants or natural medicines, one of which is miana leaves. Miana leaves are known by the public to have many uses, especially as medicine to treat various diseases. It can neutralize toxins, lung inflammation, digestive disorders, and inhibit bacterial growth **(Yuniarti, 2008)**. Miana leaves have antibacterial properties so they can be used to treat several diseases caused by bacteria **(Rahmawati, 2008)**, including inhibiting the growth of *Vibrio* sp. **(Basir *et al*, 2020; Basir *et al*, 2023)** and treating *Vibrio* sp. infection in shrimp **(Basir *et al*, 2024)**. However, the use of miana leaves in feed is not yet known with certainty concerning its ability to increase the immune system (immune) of the tiger prawns. Therefore, the aim of this research was to identify the components of the compounds in miana leaves and to assess their effects on shrimp immune parameters

MATERIALS AND METHODS

1. Extraction

Extraction was carried out by preparing simplicia (miana leaves) in flour form. Miana leaves come from Tana Toraja, South Sulawesi, weeded and washed then baked in the oven at 70ºC until dry. They were powdered and dissolved using 96% ethanol with a ratio of 1:5 w/v followed by stirring for 10 minutes and leaving for 1 x 24 hours. The solution was filtered using Whatman number 1 filter paper. The dregs were soaked again in 96% ethanol with the same solvent ratio and time. This treatment was repeated 3x or until the solution faded.

2. Gas chromatography mass spectrometry (GC-MS) analysis

Analysis of the bioactive compounds in miana leaves was performed using Gas Chromatography-Mass Spectrometry (GC-MS) with the GCMS Ultra QP2010 Shimadzu

instrument. The instrument conditions were as follows: injector temperature set at 250°C in splitless mode, with a pressure of 76.9 kPa, flow rate of 14 mL/min, and a split ratio of 1:10. The ion source and interface temperatures were 200 and 280°C, respectively. The solvent cut time was set to 3 minutes, and the mass-to-charge ratio (m/z) range was 400-700.

The column used was an SH-Rxi-5Sil MS, with a length of 30m and an inner diameter of 0.25mm. The initial column temperature was set at 70°C, with a hold time of 2 minutes. The temperature was then ramped to 200°C at a rate of 100°C/ min, and finally to 280°C at a rate of 50°C/ min, with a hold time of 9 minutes. The total analysis time was 36 minutes. Chromatogram data were analyzed using the NIST 17 and Wiley 9 libraries (SOP for the Ujungpandang Polytechnic Chemical Laboratory).

3. Test feed preparation

Test feed was made by mixing miana leaf extract with commercial feed at 0, 10, 20, and 40g/ kg feed. Miana leaf extract was added to the feed by spraying the extract evenly onto the surface of the feed and stirring until homogeneous. After the air had dried, Progol adhesive was evenly sprayed over the entire surface of the feed to coat it and to help the extract adhere to the feed.

4. Probational period

Each experimental aquarium was stocked with 10 tiger prawns, each weighing approximately 4 grams. The test feed was provided at a rate of 8% of the shrimp's body weight per day. Water conditions were maintained by changing the water daily.

5. Measurement of immune response

5.1. Total hemocytes

Total hemocytes (THC) were measured following the method of **Huynh *et al.* (2018)**. A 0.1mL sample of hemolymph, to which an anticoagulant was previously added, was counted using a hemacytometer under a microscope with 400x magnification. The total hemocytes were calculated using the following formula:

$$THC = \text{number of cells counted} \times (\text{vol. hemacytometer})^{-1} \times \text{dilution}$$

5.2. Observation of phagocytic activity

Phagocytic activity (PA) was measured following the method of **Chotigeat *et al.* (2004)**. A 0.1mL sample of hemolymph was diluted with EDTA (1:1 v/v). To this, 0.2mL of bacteria was added, and the mixture was incubated for 30 minutes at 30°C. A 5µL sample of the mixture was then placed on a glass slide, air-dried, and soaked in 2.5% glutaraldehyde for 20 minutes. The slides were washed with 0.85% NaCl, dried again, and stained with Giemsa stain for 20 minutes. Observations were made under a light microscope at 400x magnification. Phagocytic activity was calculated based on the percentage of cells exhibiting phagocytosis, as described by **Cheng *et al.* (2005)**:

$$PA\ (\%) = \frac{\text{Phagocytic cells that carry out phagocytic activity}}{\text{The number of cells observed}} \times 100$$

RESULTS

1. GCMS test results for miana leaf extract

The GCMS test results for miana leaf extract are exhibited in Fig. (1), displaying the percentage of different areas. Bulleted lists look like this:

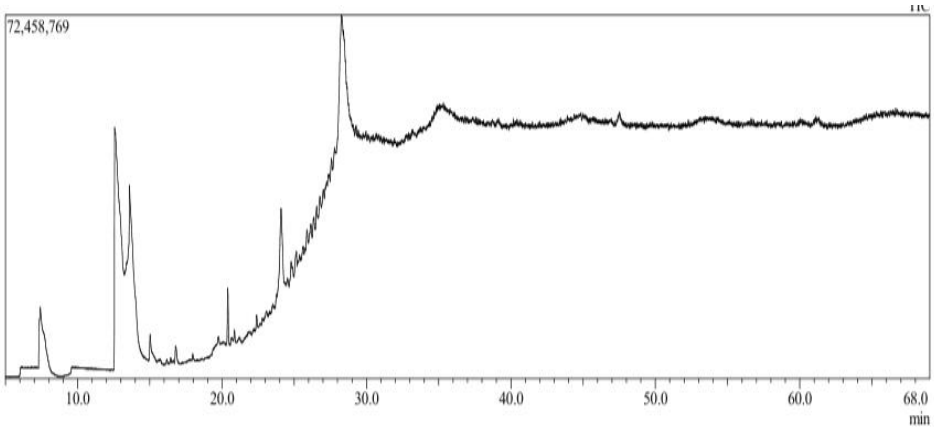

Fig. 1. GCMS results for purple miana leaf extract (*Coleus scutellarioideas*)

Table 1. Gas chromatography-mass spectrometry (GC-MS) test results for miana leaf extract

Peak	R.T.	Area Pct	Library/ID
1	7.423	3.34	1,1-Difluoroethylene-2,2-D2
2	7.650	0.33	Ethane,1-chloro-1-fluoro-(CAS) 1-chloro-1-
3	7.692	1.51	2-(Methylthio) ethanol (CAS) 2-hydroxyethyl
4	12.570	3.38	Methylsulfidtiole
5	12.625	21.13	Carbamic acid, methyl ester (CAS) methyl car
6	13.217	0.85	Propane, 1-nitro-(CAS) 1-nitropropane
7	13.267	2.09	2,2,4,4-D4-Cyclobutanol
8	13.375	2.83	2,3-Butanediol, dinitrate (CAS) 1,2-dimethyl
9	13.594	16.16	4(5H)Thiozolone, 2-amino- (CAS) psiodothia
10	14.300	0.19	1,3,5 -Trioxane, 2,4,6-trimethyl-(CAS) paraldo
11	14.571	0.14	2-exetanone, 4-methylene- (CAS) Diketene
12	15,025	0.46	2-propane (CAS) Acetone
13	16.786	0.13	Acetic acid, methyl ester (CAS) methyl acetate
14	19,751	0,13	Peroxide, bis (1-methylethyl (CAS) isopropyl
15	20.400	0,89	Hexane (CAS) n-hexane
16	20.625	0,23	Acetic acid (CAS) ethylic acid
17	20.861	0.25	Hexane (CAS) n-hexane
18	21.203	0.18	Tricyclo[4.4.0.0.3,8]deca-4,9-diene
19	22.213	0.10	Benzene, (1-methyl-1-propenyl)-, (Z) (CAS)
20	22.399	0.28	2-Pentanone (CAS) methyl propyl ketone
21	23.086	0.18	Benzene, 2-butenyl- (CAS) 1-phenyl-2-butene
22	23.507	0.24	1-Phenyl- 2-butene-1-ol

23	23.767	0.19	Benzene, 2 butenyl-(CAS) 1-phenyl-2-butene
24	24.083	3.67	N-(Tosilmethyl) formamide
25	24.525	0.14	Benzene, 2-butenyl-(CAS) 1-phenyl- 2-butene
26	24.784	0.49	Acetic acid -butyl ester (CAS) n-butyl acetate
27	24.892	9.17	Benzene, 2-butenyl-(CAS) 1-phenyl- 2-butene
28	25.150	0.54	Butanoic acid, ethyl ester (CAS) ethyl butirate
29	25.233	0.14	Acetic acid, [(2-methyl propyl) thio]- (CAS)-2-
30	25.366	0.17	IH-Indene, 2,3-dihydro- 1 - methyl -(CAS) 1-M
31	25.628	0.28	Benzene, (2 methyl-1-propenyl) -(CAS) 2-methyl
32	25.875	0.45	3- Phenylbut - 1- ene
33	26.153	0.53	3- Phenylbut - 1- ene
34	26.366	0.43	Benzene, (1- methyl -1-propenyl)-, (Z)- (CAS)
35	26.557	0.51	Benzene, 2-ethenlyl - 1,3 - dimethyl - (CAS) 2,6
36	26.771	0.85	Benzene, 1- methyl -2- (2-propenyl) -(CAS) o-A
37	27.032	0.79	Benzene, 1- ethenlyl-4-ethyl -(CAS) p Ethylstyr
38	27.150	0.36	I - Hexen-3-ol,5-nitro- I- phenyl -, (R
39	27.254	0.62	I (2H)-quinulinecarboxylic acid, 2.
40	27.377	1.01	Benzene, (2-methyl-1-propenyl)- (CAS) (2-methyl)
41	27.575	1.01	Benzene, 1-methyl-2-(2-propenyl)-(CAS) o-A
42	27.792	1.52	Benzene, (2-methyl-2-propenyl)-(CAS) methan
43	28.277	20.50	Cyclotrisiloxane, hexamethyl- (CAS) 1,1,3,3,5
44	28.950	0.90	Benzene, (2-methyl-2-propenyl)-(CAS) methan
45	29.050	0.56	1-Phenyl-2-buten-1-ol
46	29.133	0.20	1-Bromo-1,4,4A,5,8,8A-hexahydro-
47	29.279	0.56	Benzene, 1,4-dichloro- (CAS) p-dichlorobenzene
48	29.450	0.10	Benzene, 1-ethyl-4-ethyl- (CAS) p-ethylstyrylacetic
49	29.742	0.13	5-Methyl-6-phenyltetrahydro-1,3-oxazine-2-
50	29.966	0.16	Benzeneethanol, alpha.,alpha.-dimethyl-,acet
51	30.133	0.13	Benzeneethanol, alpha.,alpha.-dimethyl-,acet
52	30.668	0.13	Benzene, 2-ethenyl-1,3-dimethyl-(CAS)2,6-D
53	30.861	0.13	Benzene, 1-isopropenyl- 1 - methyL-
54	33.151	0.18	5-Mercapto-2-tert-butoxythiophe
55	33.732	0.19	
56	33.900	0.16	1,3,5-Ethanylylidene-2-thiacyclobuta[cd]penta
57	34.516	0.10	Bicyclo[4.2.1]nona-2,4,7-trien, 7-eth
58	34.617	0.10	3-Butenoic acid, 4-phenyl- (CAS) styrylacetic
59	34.683	0.15	2-Butenoic acid, 4-phenyl-, ethyl ester (CAS)
60	34.875	0.31	Benzene, (1-cyclopropyl-1-methylethyl- (CAS)
61	35,775	0.11	5-Methyl-6-phenyltetrahydro-1,3-oxazine-2-th
62	36.633	0.10	Cyclohexane, 1-ethynyl-1-isocya
63	36.788	0.097	Benzene, 2-ethenyl-1,3-dimethyl-(CAS) 2,6-
64	37.333	0.15	10-Keto-9-amino-bicyclo-(6.4.2) dec-

65	37.850	0.14	1-Hexen-3ol, 5-nitro-1phenyl-, (r*
66	38.692	0.18	2-Propanamine, N-(phenylmethylene)- (CAS)
67	39.149	0.17	6-(2-Phenyl-1-cycloprpanecarbo
68	39.900	0.23	1H-Indene, 1-ethyl-2,3-dihydro- (CAS) 1-Ethy
69	40.179	0.15	3H-3-Benzazipine-3-carbonylic acid, decahyd
70	40.308	0.13	Ethanimidothiole acid, 2-(dimethylamino)-N-
71	40.427	0.19	4-Methil-1-indanone
72	40.655	0.15	Benzeneethanol, alpha.,alpha.-dimethyl-,acet
73	41.483	0.09	1,2-trimethylene-8,9,10-trinorborna-2(3'),5-dic
74	43.492	0.15	
75	45.004	0.33	4-Phenylpent-3-en-2-one
76	45.450	0.11	1,2-O-Isopropylidene-alpha-d-glu
77	47.408	0.17	Cyclopropan, 1-methyl-1-phenyl-
78	47.499	0.36	Ditziridinone, (1,1-dimethylethyl) (1,1-dimethyl
79	47.642	0.11	1,3-Dioxolane, 2,4,5-trimethyl-2phenyl- (CAS)
80	48.148	0.11	1,2-trimethylene-8,9,10-triborna-2(3'),5-di
81	48.573	0.20	Tetralin, 1,4-diethyl-, cistrans
82	52.277	0.11	Benzenepropanol, alpha.-methyl-, acetate (CAS
83	52.608	0.13	2-(2-Oxobenzopyrrolidin-3-yliden
84	52.892	0.10	1H-Indene, 2,3-dihydro-5-methyl- (CAS) 5-M
85	53.475	0.09	4-(2-Hydroxyetylamino)-3-nitro
86	54.269	0.10	Azulene, 1,2,3,3a-tetrahydro- (CAS) tetrah
87	55.408	0.10	Cis-3-Undecene- 1,5-diyne
88	59.076	0.09	Benzene, (1-propyl-1-nocenyl)- (CAS) 4-PHE
89	60.011	0.09	1H-Indene, 1-hesadecyl--2,3, -dihydro- (CAS)
90	62.000	0.11	Cinnamyl N-phenilforminidate
91	63.917	0.11	Pentanoic acid, 4-oxo-, methyl ester (CAS) M
92	64.258	0.11	1,2-trimethylene-8,9,10-trinoborna-2(3'),5-di
93	64.417	0.12	10-Heneicosene, 11-phenyl-(CAS) 11-phenyl-
94	65.121	0.14	Androstan-17-one, 3-(acetyloxy)-
95	65.305	0.30	Benzene, 1-heptenyl- (CAS)1-phenylhep
96	65.567	0.15	Benzenecacetaldehyde, alpha. - (2-methylprupy
97	66.270	0.10	Tricyclo(4.2.1.12,5ldeca-3,7-diene-9,10-dio
98	66.450	0.12	Cabomie acid, (alpha. -methylbenzyl)-, 1-ethy
99	67.575	0.13	Benzene, 2-ethenyl-1,3-dimethyl- (CAS) 2,6-
100	68.727	0.12	Benzenepropunol gurrma. - methyl- (CA) 1-

Based on the gas chromatography mass spectrometry test (Fig. 1), there are 60 possible bioactive components resulting from the GC-MS test (Table 1). There are 3 peaks with the highest percent area, namely peak 5 with an area percent of 21.13 consisting of the active compound carbamic acid, methyl ester (CAS) methyl carbamate, a component of this compound. Meanwhile, peak 9 with a percent area of 16.16 consists of 4(5H)-Thiazolone, 2-amino- (CAS) Pseudothiohydantoin, and peak 43 with a percent area of 20.50 consists of the compounds Cyclotrisiloxane, and hexamethyl- (CAS) 1, 1,3,3,5,5-hexamethyl-cyclohexaxyloxane.

2. Total hemocytes

The total hemocyte of the tiger prawns consuming feed with the addition of miana leaf extract during 40 days of cultivation is shown in Fig. (2).

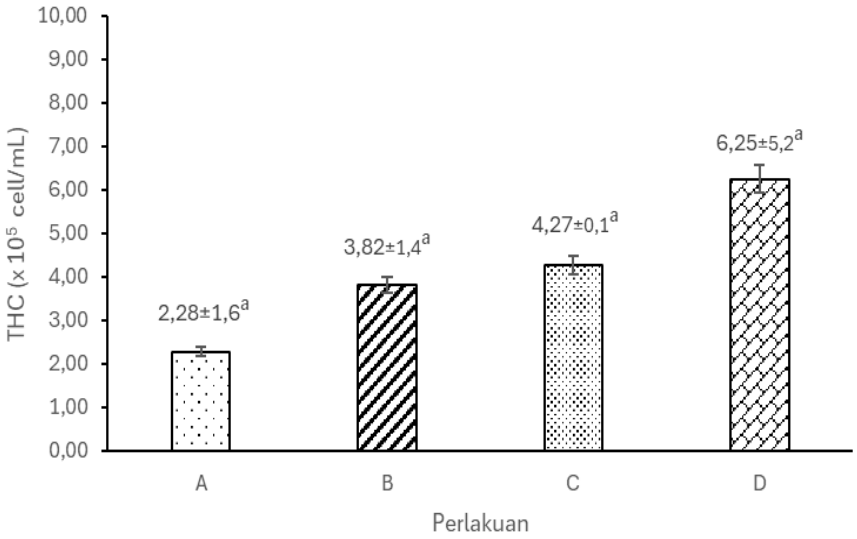

Fig. 2. Total hemocytes of the tiger shrimp for 40 days

Total hemocytes in the tiger prawns showed an increase in value along with increasing the concentration of miana leaf extract in the feed. Based on statistical analysis, it showed that the addition of miana leaf extract to feed had no significant effect on increasing the total tiger prawn hemocytes ($P > 0.05$). However, the highest total hemocytes were found in the treatment with the highest addition of feed extract, namely 40g/ kg feed.

3. Phagocytic activity

The percentage of phagocytic activity of the tiger prawn hemocytes against bacteria is exhibited in Fig. (3).

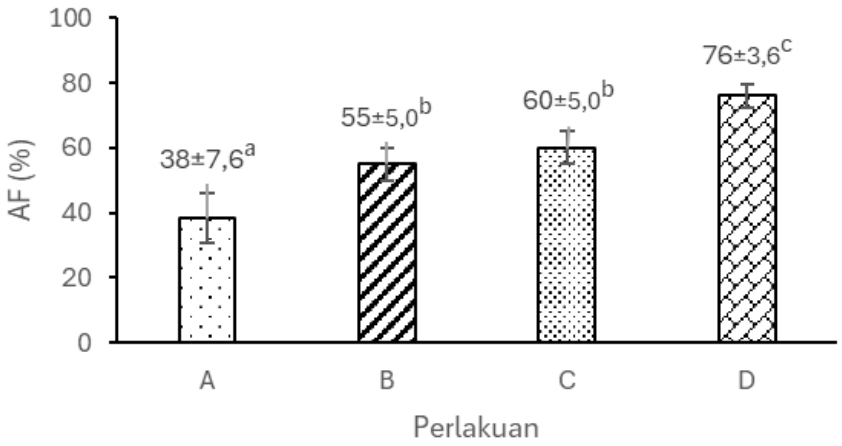

Fig. 3. Phagocytosis of the tiger shrimp hemocytes against bacteria

Based on Fig. (3), it can be seen that the phagocytic activity of blood cells in the tiger prawns is in line with the increasing concentration of miana leaf extract in the feed. The results of statistical analysis showed that the treatment had a significant effect ($P< 0.05$) on the phagocytic activity of the tiger prawn blood cells. The highest activity was found in the treatment with the addition of miana leaf extract at a dose of 40g/ kg feed. Meanwhile, the lowest phagocytic activity was found in the feed treatment without the addition of miana leaf extract. The average phagocytosis value of the 40g/ kg feed treatment was different from all treatments (0, 10, and 20g/ kg feed).

The phagocytic activity of hemocytes against bacteria is characterized by cells that appear thickened and filled (Fig. 4).

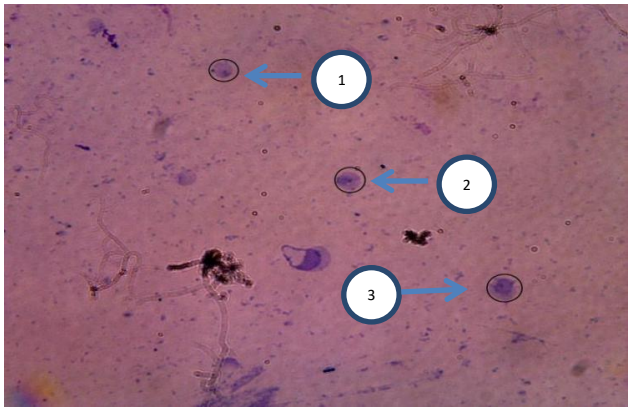

Fig. 4. Blood cells perform phagocytosis (1,2,3)

The activity of blood cells carrying out phagocytosis (Fig. 4) was observed under an electron microscope with a magnification of 400 times. The highest number of phagocytosed cells was found in the treatment with the addition of 40g/ kg of miana leaf extract.

4. Survival rate

Through this experiment, it was discovered that, the highest survival rate for tiger prawns was found in the treatment with the addition of 40g/ kg miana leaf extract, namely 86.67%. Meanwhile, survival was lowest in the feed treatment without the addition of miana leaf extract (0g/ kg). Based on statistical tests, results show that the treatment with the addition of extract to the feed has a significant effect ($P< 0.05$) on the survival of the black tiger prawns.

Table 5. Average survival rate for tiger prawns during 40 days of cultivation

Treatment (g/kg)	Test			Average (%) ±SD
	1	2	3	
0	50	70	60	60,00[a]±10
10	90	80	70	80,00[a]±10
20	70	90	70	76,67[a]±11,55
40	90	90	80	86,67[ba]±5,77

Different superscripts following numbers in the same column indicate different influences.

DISCUSSION

1. GCMS test results for miana leaf extract

Carbamic acid is the most abundant compound found in the GC-MS analysis of purple miana leaf extract. According to **Fernandes *et al*. (2016)**, carbamic acid possesses antimicrobial and antifungal properties, indicating its bioactivity. This suggests that miana leaves have potential as an immunostimulant. The second most abundant compound in miana leaves is Cyclotrisiloxane, a compound known for its antibacterial, anti-inflammatory, and anticancer activities **(Jung & Shin, 2021; Muthukrishnan *et al*., 2022)**. The presence of Cyclotrisiloxane further supports the potential of miana leaves as an immunostimulant, as it helps reduce inflammation and fight cancer.

The bioactive compound content in miana leaves, shown in Table (1), highlights their potential use as both an immunostimulant and a medicine. Miana leaves have been widely used in traditional medicine due to their healing properties, especially for bacterial infections. Their bioactive content can enhance overall health **(Salimi, 2021)**. *In vitro* tests have confirmed their antibacterial properties **(Basir *et al*., 2024)**, with a bacterial inhibition zone of 28mm for miana leaf extract, compared to 30mm for antibiotics like Oxy. This indicates the antibacterial potential of miana leaves. Furthermore, miana leaf extract has been tested on vaname shrimp infected with *Vibrio harveyi*, resulting in the shrimp recovering from disease symptoms after a 10-minute immersion for four days

(Basir *et al.*, 2023). These studies demonstrate the efficacy of the bioactive compounds in miana leaves.

2. Total hemocytes

The tendency for an increase in total hemocytes in the tiger prawns fed commercial feed supplemented with miana leaf extract (Fig. 2) demonstrates the ability of miana leaves to stimulate the production of hemocytes. This effect is attributed to the bioactive compounds in miana leaves (Table 1). Cyclotrisiloxane, which acts as both an anti-inflammatory **(Rahmawati, 2008)** and antibacterial agent **(Rahmawati, 2008; Yuniarti, 2008; Jung & Shin, 2021; Muthukrishnan *et al.*, 2022; Basir *et al.*, 2023)**, plays a protective role in cells and tissues.

The anti-inflammatory properties of miana leaves help reduce tissue damage caused by microorganisms, thereby supporting the shrimp's immune system. Inflammation can be triggered by microbial invasion, ischemia, antigen-antibody reactions, or physical injuries **(Anggraeny & Pramitaningastuti, 2016)**.

The potential of miana leaves as an immunostimulant was clearly demonstrated in this experiment. The bioactive compounds in miana leaf extract stimulate the hemocytes to degranulate and release immune proteins, such as ß-glucan-binding protein (ßG-BP), peptidoglycan-binding protein (PG-BP), lipopolysaccharide-binding protein (LPS-BP), coagulation factors, prophenoloxidase-related factors, and antimicrobial substances (penaedin, lectin). These proteins play a key role in immune responses, particularly phagocytosis **(Darwantin *et al.*, 2016)**.

3. Phagocytic activity

The highest concentration of miana leaf extract (40g/ kg feed) produced the best hemocyte phagocytic activity, indicating the ability of miana leaves to stimulate hemocytes to perform phagocytosis. Phagocytosis occurs when foreign bodies, such as bacteria or viruses, enter the shrimp’s body. The ability of blood cells to perform phagocytosis is influenced by the shrimp's immune system. Miana leaves contain active compounds such as carbamic acid, acetic acid (vinegar), and butanoic acid (butyric acid), shown in Table (1), which support the health of blood cells and tissues, thereby enhancing their ability to perform phagocytosis.

Butyric acid is a primary energy source for colon cells and plays a role in natural immunity, apoptosis induction, and the regulation of body water and electrolytes. It is also an important metabolite in fat, carbohydrate, and protein breakdown **(Karimi & Vahabzadeh, 2014)**. Miana leaves also contain high levels of rosmarinic acid, which acts as an antioxidant, helping to improve organ function, particularly in boosting shrimp immune responses.

Acetic acid (vinegar) is traditionally used to combat infections. Studies suggest that vinegar can improve conditions like obesity, diabetes, cardiovascular issues, cancer, and microbial infections **(Samad *et al.*, 2016)**. The presence of acetic acid in miana leaves

contributes to the enhancement of phagocytic activity and supports other immune parameters.

Phagocytic activity is a critical nonspecific defense mechanism in invertebrates, helping to protect shrimp from pathogens **(Rengpipat *et al.*, 2000)**. The addition of miana leaf extract significantly increases hemocyte phagocytic activity, suggesting that miana leaf bioactive compounds positively influence shrimp immunity.

4. Survival rate

The addition of miana leaf extract to feed significantly improved the survival rate of the black tiger prawns compared to the control group. This positive result indicates that miana leaf extract enhances feed quality, contributing to better shrimp health **(Darwantin *et al.*, 2016)**. Improved shrimp health leads to better survival and growth rates, as the enhanced immune system enables the prawns to adapt to fluctuating environmental conditions. A stronger immune system increases the likelihood of shrimp survival.

Previous studies on immunostimulants, such as Zoothamnium Penaeid membrane protein, showed a significant increase in survival rates—from 26% to 83% in vaname shrimp. When applied to the tiger prawn ponds, survival rates also increased from 16% to 78% **(Gustrifandi, 2013)**.

Miana leaves are known for their high antioxidant content, with an IC50 value ranging from 33.768 to 48.04ppm **(Afifah *et al.*, 2015; Giuliana *et al.*, 2015)**. Antioxidants protect shrimp from oxidative stress and free radical damage, supporting overall health and contributing to a higher survival rate. These findings highlight the importance of miana leaves in enhancing shrimp health and resilience.

CONCLUSION

The research results lead to the following conclusions:

1. Purple miana leaf extract (*Coleus scutellarioides* L. Benth) contains 100 bioactive compounds, which have the potential to act as immunostimulants.
2. Miana leaves contain bioactive compounds that enhance total hemocytes and phagocytosis, significantly supporting the increased survival of tiger prawns.

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