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The Use of Purik Leaf Extract (*Mitragyna speciosa*) on Hematological and Histopathological Profile of Vannamei Shrimp (*Litopenaeus vannamei*) on *Vibrio Parahaemolyticus* Infection

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Abstract: The purpose of this study was to analyze the optimal dose of Purik leaf extract (*Mitragyna speciosa*) which has antibacterial compounds to treat vannamei shrimp *(Litopenaeus vannamei*) that infected by *Vibrio parahaemolyticus.* The method used in this research is experimental study with Completely Randomized Design (CRD). The results form GC-MS analysis of purik leaf extract showed that the most abundance compounds were *Quinic acid* and *Mitragynine* from flavonoid and alkaloid class. Total Hemocytes Count (THC) after purik leaf treatment on vibrio infected vannamei shrimp showed that extract at 250 mg/L dose showed the best result with 8.03x10^6 cells/ml. The Differential Haemocyte Count *(*DHC*)* after treatment showed that the most effective dose is 250 mg/L with 20.33% hyaline, 23.67% semigranular cells, and 56% granular cells. Based on histopatology observation of vannamei hepatopancreas after treatment showed that the best dose is 250 mg/L with 1.20% haemorrhage, 1.20% necrosis, and 1.40% cell inflammation. Purik leaf extract which contains many compounds can be useful as an antibacterial to inhibit and destroy *V. parahaemolyticus* bacteria and proven to treat vannamei shrimp *(L. Vannamei*) that infected by *V. parahaemolyticus.*

Keywords: Alkaloids; Flavonoids; *Litopenaeus vannamei; Mitragyna speciosa; Vibrio parahaemolyticus*

Introduction

Vannamei shrimp farming (*Litopenaeus vannamei*) is a rapidly growing industry in Indonesia (Octovianus et al., 2023). Vannamei shrimp farming especially in intensive system experience the attack of various plague disease including vibriosis (Rahmi et al., 2023). Vibriosis is a disease caused by vibrio bacteria kind and one of them is *Vibrio parahaemolyticus*. The attack of *V. parahaemolyticus* cause a number of syndrome in vannamei shrimp cultivation including Acute Hepatopancreatic Necrosis Disease (AHPND) and Early Mortality Syndrome (EMS) that cause death up to 100% on vannamei shrimp cultivation (Quang et al., 2020). In controling and curing the shrimp disease usually done with usage of chemical materials and antibiotics (Anarkhis et al., 2023). Treatment with chemical materials and antibiotics have own weakness that can raises negative impact for waters environment, raises pathogens resistance and residues of antibiotics. The use of herbals or phytopharmaca is possible solution for overcome problem (Marwulan et al., 2023).

Purik Leaf (*M. speciosa*) is one of the herbal plant that can become a potential candidate for antibacterial material of vannamei vibriosis treatment. Purik leaf own quercetin compound which is flavonoids class that play an active role in the antibacterial process (Hotimah et al., 2021). Purik leaf also has a very rare alkaloids compound

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that is Mytragynine. Mitragynine has general function as analgesic, antitussive, antidiarrheal, adrenergic, antimalarial (Hassan et al., 2013), antibacterial and antinociceptive (Juanda et al., 2019). THC and DHC parameters can be used as a reference to see the health condition of vannamei shrimp (Mahasri et al., 2018). Based on studies on the potential of *M. speciosa* as an antibacterial, many references have been carried out, but its use as an alternative antibacterial against *V. parahaemolyticus* bacteria are not yet optimal, thus, it is necessary to carry out further research and study in detail the active compound content of *M. speciosa* which is useful as an antibacterial.

Method

Research Materials

The materials used in the extraction activities are purik leaves *(M. speciosa) and* solvents for maceration namely methanol with pro-analysis (PA) quality, *V. parahaemolyticus* bacteria, EDTA, and giemsa.

Draft Study

The design in this study was Completely Randomized Design (CRD). The treatment used in this study was five different doses, control positive, control negative and test as much 3 time. The treatments are as follows:

Treatment A = Treatment of bacterial infection by leaf extract (*M. speciosa*) 150 ppm
Treatment B = Treatment of bacterial infection by leaf extract (*M. speciosa*) 200 ppm
Treatment C = Treatment of bacterial infection by leaf extract (*M. speciosa*) 250 ppm
Treatment D = Treatment of bacterial infection by leaf extract (*M. speciosa*) 300 ppm
Treatment E = Treatment of bacterial infection by leaf extract (*M. speciosa*) 350 ppm
treatment K- = Treatment of bacterial infections without giving extracts
treatment K+ = Treatment of bacterial infection by giving antibiotics

Research Procedure

Making Extract of Purik Leaves (M. speciosa)

Making extract of purik leaves begin with taking leaves from fresh purik plants (*M. speciosa)*. The leaf samples then washed and dried in the sun. The dried leaves were weighed and then blended. Furthermore, 500 grams purik leaf powder was macerated with 96% methanol as much as 1500 ml for 3 x 24 hours. Maserate is filtered with filter paper. Purik leaf extract was evaporated using *a rotary evaporator* with a temperature of 40 ◦C (Lolodatu et al., 2019).

Gas Chromatography-Mass Spectrometry (GC-MS)

Gas Chromatography Mass Spectrometry (GC-MS) is a gas chromatography technique used in conjunction with mass spectrometry. Gas chromatography is used to search for volatile compounds under high vacuum and low pressure while heating. Mass spectrometry is used to determine molecular weight, molecular formula and produce charged molecules (Ari et al., 2016).

The preparation of purik leaf extract uses the maceration or soaking method. Then it was put into a micro tube containing 0.5 g of powder and 1.5 ml of ethanol solvent, then vortexed for 1 minute, then centrifuged for 3 minutes at 9000 rpm. The supernatant formed was continued for GC-MS testing. The time is set for 60 minutes with an injector temperature of 260°C, detector 250°C, and column 325°C. The carrier gas used is helium gas as the carrier at a constant flow rate of 1 ml/min. The identification process using the GC-MS tool to produce several bioactive compounds can be seen from the peaks of the chromatogram as identification of data from chromatography and mass spectrometry (MS) results seen from the mass spectrum with the molecular weight of each bioactive compound (Khair et al., 2017).

Total Haemocyte Count (THC)

Total Haemocyte Count (THC) is calculated using a haemocytometer with microscope help with 400x magnification (Victor et al., 2023), calculated with following formula.

$$\text{THC} = \sum \text{Blood cell} \times \frac{1}{0.1} \times \frac{1}{\frac{\sum \text{Blood}}{\sum \text{Blood} + \text{anticoagulant}}} \quad (1)$$

Differential Haemocyte Count (DHC)

Observation cell amount of differential hemocytes (hyaline, semigranular, and granular) the percentage based on criteria morphology with use microscope light 1000X magnification (Victor et al., 2023), calculated with following formula.

$$\text{DHC} = \frac{\sum \text{Hyaline cells/Granule}}{\sum \text{Observed cells}} \times 100\% \quad (2)$$

Histopathology Observation

Taking tissue on hepatopancreas organs are made on all shrimp treatment group. Tissue that has been taken cleaned with aquadest. Organ tissue then put in to film bottles that containing 10% formalin, then followed by making histopathology specimen. Hitopathology specimen results then observed under microscope and done a scoring tissue damage (Andayani et al., 2018).

Results and Discussion

GC MS Analysis of Purik Leaf Extract (Mitragyna speciosa)

Results of GC-MS analysis of extracts leaf purik show that there are 52 peaks and 270 possibilities component compounds are extracted successfully from methanol solvent. The component can be seen in Table 1.

Table 1. Results of GC-MS Tests on Purik Leaf

Peak	Real Time	Hits 1	Hits 2	Hits 3	Ret. areas
1	2.476	*Propanal*	*2.3-dihydroxy-*	*alpha beta . . - Dihydroxypropional - dehyde . DL-GLYC*	0.97
2	2.671	*2-Furanmethanol*	*Furfuryl alcohol L*	*alpha.- Furfuryl alcohol*	0.50
3	3025	*3. beta.- hydroxy-(20s)-20-n*	*N-dimet hylamino-4.4*	*14. alpha . -trimethyl-9. 19-cycl*	0.64
4	3.324	*2-Hydroxy-2-cyclopenten-1-one*	*2 -Cyclopenten-1-one*	*2-hydroxy-*	0.52
5	3.504	*2.5-Furandione*	*dihydro-3-methylene*	*Itaconic anhydride*	0.27
6	3.894	*4H-Pyran-4-one*	*2.3-dihydro-3*	*5-dihydroxy-6-methyl-*	1.80
7	4.109	*4-Hydroxy-*	*2-methyl-*	*2- cyclopentenone*	1.43
8	4.658	*N'-Dimethyl-piperazine*	*Piperazine. 1.4-dimethyl-*	*Lupetazine*	0.88
9	4.866	*Hydroxy dimethyl furanone*	*4-hydroxy-2.5-dimethyl-3(2h)-furanone*	*Furaneol*	0.56
10	5.158	*4H-Pyran-4-one*	*2-hydroxy-3-methyl -(CAS)*	*2-Hydroxy-3-methyl-4-pyrone*	0.44
11	5.249	*2.3-Dihydro-*	*5-hydroxy-*	*6-methyl-4H-pyran-4-one*	0.20
12	6.068	*2.3-Dihydro-3*	*5-dihydroxy-*	*6-methyl -4H-pyran-4-one*	1.42
13	6.200	*dl-Threitol*	*1.2.3.4-Butanetetro l*		0.56
14	6.979	*1.2-Benzenediol (CAS)*	*Pyrocatechol*	*Brenzcatechin. Fourrine 6*	3.13
15	7.118	*4-vinylphenol*	*p- vinylphenol*	*1-Ethenyl-4-hydroxybenzene*	2.35
16	7.229	*2-Furancarbox-aldehyde*	*5-(hydroxymethyl)-*	*2-Furaldehyde. 5-(hydroxymethyl)-*	1.69
17	7.444	*1.2.3-Propanetriol. monoacetate*	*Acetin . mono-*	*Acetin . Acetog lycerides*	0.80
18	8042	*2.3-Dihydro-3*	*5-dihydroxy-*	*6-methyl-4H-pyran-4-one*	0.80
19	8.257	*1.2-Benzenediol. 4-methyl-*	*3.4- Dihydroxytoluene*	*Homocatechol*	0.47
20	8.368	*2-Methoxy-4-vinylphenol*	*Phenol. 4-ethenyl-2-methoxy-*	*p- Vinylguaiacol*	2.11
21	8.862	*Phenol. 2.6-dimethoxy-*	*Pyrogallol 1.3-dimethyl ether*	*Syringol*	0.73
22	10.231	*4H- Pyrazolo[3. 4-d]pyrimidine-4-one*	*1.5-dihydro- (CAS)*	*BW 56-158. Gotax*	0.47
23	10.814	*1.6-anhydro-beta-d-glucopyranose (levoglucosan)*	*Leucoglucosan*		1.81
24	11.537	*4 - vinyl - syringol*	*2.6-di-methoxy -*	*4 - vinyl - phenol*	1.17
25	11.641	*Dodecanamide*	*N-(4-chlorophenyl)-*	*N-(4-Chlorophenyl). dodecanamide*	0.13
26	12.677	*Quinic acid*	*(1R.3R.4R.5R) -(-)- Quinic acid*	*D(-)-Quininic acid*	18.28
27	13.114	*Mome inosythol*			1.56
28	13.573	*Undecano-4-lactone*			8.78
29	13.941	*(-)- Loliolide*	*Loliolide*	*Digiprolactone . Calendin*	0.39
30	14.143	*4.5-Pyrimidine-diamine. 6-methyl-*	*Pyrimidine*	*4.5-diamino-6-methyl*	0.19
31	14.462	*4a-Methyl-4.4a*	*5.6-tetrahydro-2(3H)*	*7-indendione*	1.94
32	14.720	*Allo inositols. allo -Inositol (CAS)*	*Alloinositol*	*Inositol. allo -*	0.35
33	15.345	*Hexadecanoic acid. methyl ester*	*Palmitic acid. methyl ester*	*Uniphat A60*	1.27
34	15.748	*n- Hexadecanoic acid*	*Hexadecanoic acid*	*n- Hexadecoic acid. Palmitic acid*	2.52
35	17.061	*9.12.15-Octadecatrienoic acid*	*methyl ester. (Z.Z .Z)-*	*Methyl linolenate*	1.45
36	17.172	*2-Hexadecen-1-ol*	*3.7.11.15-tetramethyl-*		0.38
37	17.263	*Methyl 16-methyl-heptadecanoate*			0.15
38	17.471	*9.12.15-Octadecatrienoic acid*	*Linolenic acid*	*Industry 120*	7.21
39	17.645	*Octadecanoic acid (CAS)*	*Stearic acid*	*acid. Vanicol*	0.58
40	20.556	*Hexadecanoic acid*	*2-hydroxy-1-(hydroxymethyl) ethyl ester*	*Palmitine . 2-mono-*	0.67
41	21.119	*2.5bis(dimethyl- chlorosilyl)furan*			0.84
42	21.946	*E.Z -1.3.12-Nonadecatriene*			0.22
43	22009	*(7R.8S)-cis-anti-cis-7*	*8-Epoxytricyclo*	*dodecane*	0.25
44	23.009	*2.6.10.14.18.22-Tetracosahexaene*	*2.6.10.15.19.23-hexamethyl-.*		0.75
45	26.629	*dl -.alpha .-Tocopherol*	*alpha. - Tocopherol*	*Vitamin E*	0.83
46	27.046	*Corynantheidine (allo)*	*Corynant heidine*	*Epidihydrocorynantheine*	0.76

Peak	Real Time	Hits 1	Hits 2	Hits 3	Ret. areas
47	28.235	*Ergost-5-en- 3.beta .-ol*			0.73
48	28.818	*1.2.5-Oxadiazol-3-amine*	*4-(4-methoxyphenoxy)-*		0.54
49	29.958	*-ethylcholest-5-en- 3.beta .-ol*			1.24
50	31.890	*Partnerqynine (allo)*	*Mitragynine*	*9-Methoxycorynant heidine*	16.71
51	32.390	4.6-Dimethoxy-2	3-diphenyl-7-(1-pyrrolin-2-yl)indole		3.24
52	32.974	*Speciogynin*	*Speciogynine*		2.33

The most abundance compound on purik leaves extract located at peak 26 and peak 50. Peak 26 with retention area 18.28% and peak 50 with mark retention area of 16.71%. Compounds found in peaks 26 and 50 namely *Quinic acid* and *Mitragynine.* According to the pubchem website that compound *Quinic acid* own synonym name *D(-)- Quinic acid and Mitragynine* own synonym name *(-)- Mitragynine picrate. Mitragynine* is the main alkaloid indole that can be found in kratom, specifically in leaves. Kratom contains more than 40 types of alkaloids which one is *Mitragynine*. *Mitergynine* has benefit as pain reliever or tratment of opioid addiction and can be antibacterial compound. According to Raini (2017), the benefit of kratom leaves that have been researched previously that is analgesic, sedative, stimulant, antidepressant, anti-inflammatory antioxidants as well as antimicrobial. The compound of leaves extract can be different based on the natural materials used, such as the type of plant, the age of the plant, the part used, and the environmental conditions the plant comes from which includes water, temperature, light, and soil (Gerrine et al., 2023).

Total Haemocyte Count (THC)

Total Hemocytes Count (THC) after purik leaf treatment showed significantly different results in several dose, but in treatment C, D, and E are not significantly different. The highest THC value obtained in treatment is treatment E (350 mg/L) with 8.15 x 10^6 cells/ml then treatment C (250 mg/L) with 8.03 x 10^6 cells/ml and treatment D (200 mg/L) with 8.01 x 10^6 cell/ml. This value show that shrimp on the third treatment in healthy condition seen from the total hemocytes obtained. According to Febriania et al. (2018), total hemocytes count (THC) of healthy shrimp with 11-12 g weight is about 1.80 ± 9.28 x 10^7 cells/ml.

Table 2. THC of shrimp before infection, after *V. parahaemolyticus* infection, and after *Mitragyna speciosa* treatment

Treatment (mg/L)	Before Infection	Infected	After Treatment
K-	9.11 ± 0.35	8.93 ± 0.45	8.89 ± 0.34 c
150	8.46 ± 0.22	6.71 ± 0.48	7.09 ± 0.15 a
200	8.33 ± 0.21	6.89 ± 0.17	8.07 ± 0.58 a
250	8.88 ± 0.18	6.94 ± 0.19	8.03 ± 0.12 b
300	8.61 ± 0.33	7.02 ± 0.43	8.01 ± 0.12 b
350	8.61 ± 0.47	6.93 ± 0.17	8.15 ± 0.18 b
K+	8.34 ± 0.43	6.45 ± 0.18	6.85 ± 0.29c

Maintenance treatment of vannamei shrimp using purik leaf extract at 250 mg/L dose showed the best result. Hemocytes is cells defense system in vannamei shrimp, which is have a role to do phagocytosis, nodulation and encapsulation. High ammount of haemocytes show that the health level of vannamei shrimp is good (Rahim et al., 2020). The increasement of THC after treatment is the impact of giving purik leaf extract as an antibacterial starting to absorb by the body of the vannamei shrimp so that the body condition is improves.

Differential Haemocyte Count (DHC)

Type of haemocyte cells observed is hyaline, granular, and semigranular cells. Based on one-way anova test, purik leaf extract treatment give significant influence to vannamei shrimp DHC value. Haemocytes consists from three differentiated type of cells based on granules in the cytoplasm of each cell that is hyaline, granular, and semi-granular. Three type of cells have a function to destroy foreign particle that enter the shrimp body through phagocytosis, encapsulation, nodules formation, and production of humoral components that stored in haemocytic granules, namely anticoagulant proteins, agglutinins, PO enzymes, antimicrobial peptides, and protease inhibitors (Munaeni et al., 2020). Phagocytosis is most common cell defense reaction and happenend together with secretion of humoral component.

Table 3. Hyaline cell of shrimp before infection, after *V. parahaemolyticus* infection, and after *Mitragyna speciosa* treatment

Treatment (mg/L)	Before Infection	Infected	After Treatment
K-	16.33 ± 0.05	17.00 ± 0.04 ^{a}	18.67 ± 0.04 ^{a}
150	14.33 ± 0.05	23.67 ± 0.04 ^{b}	23.00 ± 0.04 ^{b}
200	15.00 ± 0.02	24.33 ± 0.43 ^{b}	24.00 ± 0.14 ^{b}
250	14.67 ± 0.04	24.33 ± 0.45 ^{b}	20.33 ± 0.04 ^{ab}
300	12.67 ± 0.03	23.67 ± 0.50 ^{b}	20.67 ± 0.09 ^{ab}
350	14.00 ± 0.03	26.67 ± 0.57 ^{b}	20.33 ± 0.05 ^{ab}
K+	12.67 ± 0.01	25.67 ± 0.41 ^{b}	20.33 ± 0.09 ^{ab}

All type hemocyte cells can do phagocytosis activity, but in general hyaline cell has more active role in phagocytosis activity (Sundell et al., 2014). In phagocytosis, cell hyaline will fold and destroy pathogens or foreign particles in shrimp body (Lesmanawati, 2013). Increasing percentage of hyaline

cell in shrimp after infected is because of the shrimp self-defense to counter *V. parahaemolyticus* in shrimp body. Hyaline cells play a role in phagocytosis, where phagocytosis is the first line of defense against pathogens (Sundell et al., 2014).

Based on the results after treatment, the lowest percentage of hyaline cell indicated by three treatments C, D, and E with unsignificantly different value. The most effective dose used in this test is treatment C (250 mg/L) with 20.33% hyaline value. Decrease of hyaline cell value after treatment because the compounds contained in the purik leaf extract have antibacterial function. According to Awad & Amani (2017) herbal extract treatment that containing antibacterial and immunostimulant compound proven can increase immune response through phagocytosis activation.

Table 4. Semigranular cell of shrimp before infection, after *V. parahaemolyticus* infection, and after *Mitragyna speciosa* treatment

Treatment (mg/L)	Before Infection	Infected	After Treatment
K-	22.67 ± 0.20	22.67 ± 0.01^{a}	22.67 ± 0.01 ^{b}
150	22.33 ± 0.03	29.67 ± 0.05 ^{b}	28.00 ± 0.06 ^{s}
200	22.67 ± 0.05	30.33 ± 0.07 ^{b}	28.33 ± 0.08 ^{d}
250	22.67 ± 0.08	31.00 ± 0.10 ^{b}	23.67 ± 0.03 ^{bc}
300	23.33 ± 0.28	31.00 ± 0.02 ^{b}	25.33 ± 0.10 ^{c}
350	22.33 ± 0.05	30.33 ± 0.07 ^{b}	25.33 ± 0.04 ^{c}
K+	22.67 ± 0.10	30.00 ± 0.04 ^{b}	20.33 ± 0.08 ^{a}

Total semigranular cells in vannamei shrimp after infection show increase percentage value and after treatment show decrease percentage value. The lowest semigranular cells value obtained in treatment C which is 23.67%. This value show that active compound on the purik leaf extract is influential. According to Bussabong et al. (2021), alkaloids in purik leaf extracts as antibacterial can increase immune system.

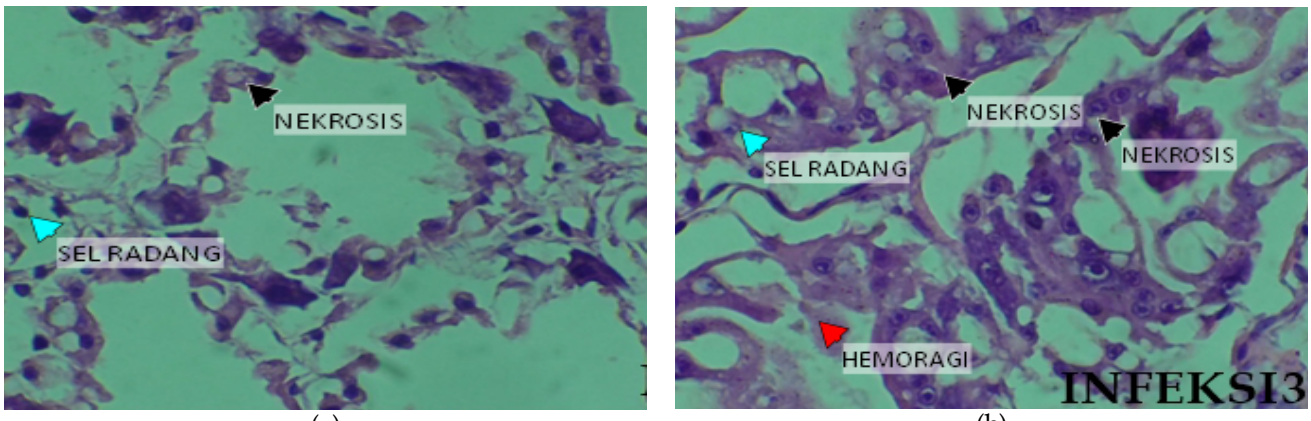
Table 5. Granular cell of shrimp before infection, after *V. parahaemolyticus* infection, and after *Mitragyna speciosa* treatment

Treatment (mg/L)	Before Infection	Infected	After Treatment
K-	61.00 ± 2.00	60.33 ± 0.57 ^{c}	58.67 ± 0.57 ^{cd}
150	63.33 ± 2.51	46.67 ± 1.52 ^{b}	49.00 ± 1.00 ^{a}
200	62.33 ± 1.52	45.33 ± 1.15 ^{ab}	47.67 ± 1.52 ^{b}
250	62.67 ± 1.52	44.67 ± 1.52 ^{ab}	56.00 ± 2.00 ^{bc}
300	64.00 ± 2.64	45.33 ± 3.05 ^{ab}	54.00 ± 2.00 ^{b}
350	63.67 ± 1.15	43.00 ± 2.00 ^{a}	54.33 ± 2.08 ^{b}
K+	64.67 ± 3.05	44.33 ± 1.52 ^{ab}	59.33 ± 1.52 ^{s}

Besides hyaline, other kind of hemocytes cells involved in shrimp immune system is granular cells. Granular cell will do degranulation at early stage of infection, then will changed become cells that have flat concentric layer and necrosis will happened. This process will kill foreign organism or at least reduce the movement and inhibit the foreign object growth (Fangli et al., 2019).

Highest percentage value of granular cells after treatment in this research is shown by treatment C with 56.00%. Treatment C granular cells value is unsignificantly different with treatment K- and K+. The highest granular cells value were also shown by treatment D and E, but three treatment C, D, and E are have unsignificantly different value. Based on this study, can concluded that the best treatment obtained in treatment C, because own a lower dose compared to treatment D and E, however can stimulate optimally on the increase of granulocytes cell in vannamei shrimp.

Histopathology Observation



(a) (b)

Figure 1. Hepatopancreas Histopathology of Vannamei Shrimp (*L. vannamei*) (a) Normal and (b) Vibriosis with magnification 400x microscope

Based on Figure 1, shows condition healthy shrimp showed a little cell tissue inflammation and necrosis, but the structure of the hepatopancreas organ still complete. Meanwhile, in the *V. parahaemolyticus* (vibriosis) infected

hepatopancreas is seen lots of damage that occurs, such as hemorrhage, cells inflammation, and necrosis. Infected shrimp hepatopancreas after done treatment using purik leaf extract as presented in Figure 2.

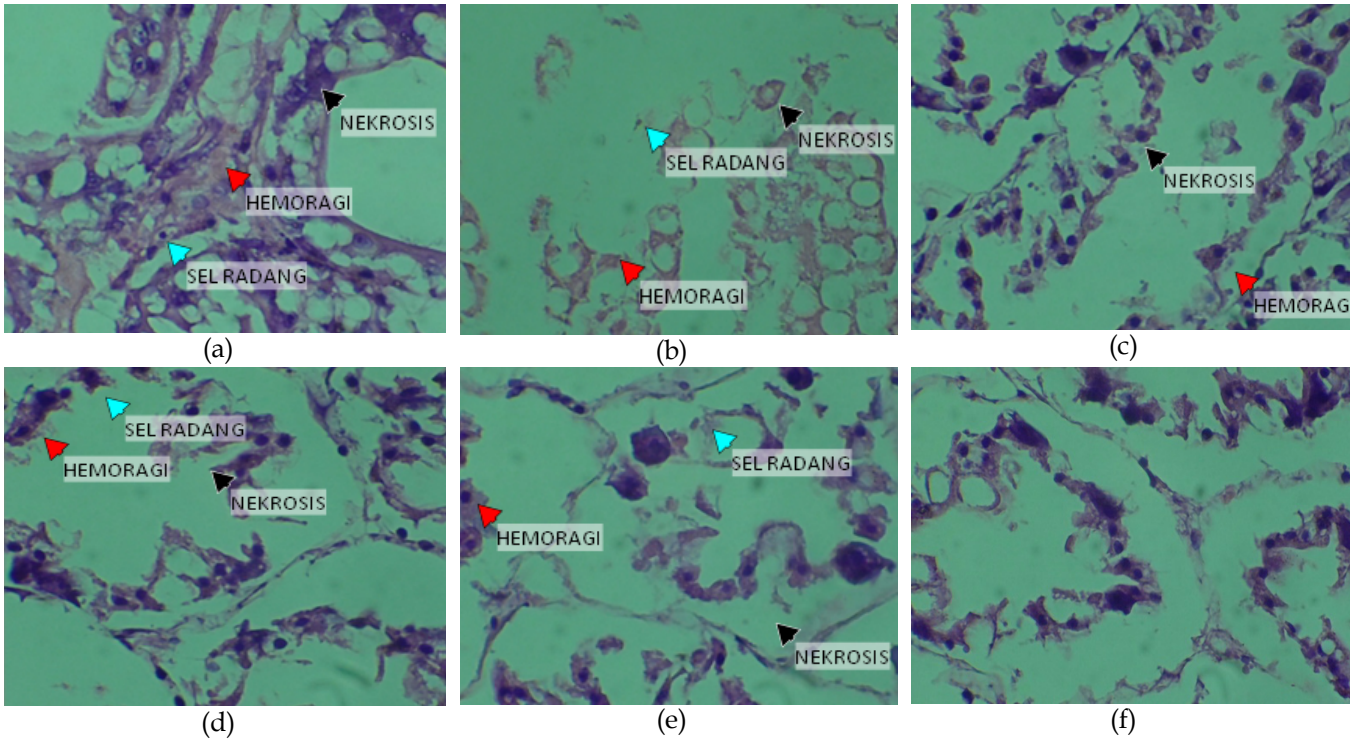


Figure 2. Hepatopancreas Histopathology Vannamei Shrimp (L. *vannamei*) Post Treatment with Magnification Microscope 400x (a) dose of 150 mg/L (b) dose of 200 mg/L (c) dose of 250 mg/L (d) dose of 300 mg/L (e) dose of 350 mg/L and (f) Tetracycline

Damage analysis to the vibrio infected hepatopancreas post treatment use extract leaf purik can be seen in Table 6. Based on one-way anova test in Table 6 obtained results that hemorrhage damage to the hepatopancreas tissue show significant result ($P < 0.05$).

Table 6. Damage Analysis of Vibrio Infected Hepatopancreas Organ Post Treatment

Treatment (mg/L)	Hemorrhage	Necrosis	Cell Inflammation
K+	1.00 ± 0.20 ^{b}	1.00 ± 0.40 ^{a}	1.20 ± 0.30 ^{a}
150	2.80 ± 0.20 ^{s}	2.20 ± 0.30 ^{s}	2.80 ± 0.20 ^{c}
200	2.40 ± 0.40 ^{d}	2.07 ± 0.70 ^{cd}	2.20 ± 0.50 ^{c}
250	1.20 ± 0.20 ^{bc}	1.20 ± 0.20 ^{b}	1.40 ± 0.40 ^{b}
300	1.27 ± 0.30 ^{bc}	1.40 ± 0.20 ^{bc}	1.40 ± 0.20 ^{b}
350	1.60 ± 0.40 ^{c}	1.60 ± 0.40 ^{bcd}	1.40 ± 0.40 ^{b}
K-	0.33 ± 0.11 ^{a}	0.33 ± 0.11 ^{ab}	0.47 ± 0.20 ^{b}

Research results show that purik leaf extract treatment can lower hepatopancreas haemorrhage in vibrio infected vannamei. The lowest haemorrhage damage is in 250 mg/L (C) treatment dose with damage value by 1.20%. The damage value for treatment C is simillar to the K+ treatment. This suspected that the compounds contained in the purik leaves have ability to regenerate and repair damaged tissue in the vannamei hepatopancreas. Quinic compound from flavonoid group suspected as anti-inflammatory substance. According to Narande et al. (2013), flavonoids can inhibit cyclooxygenase or lipooxygenase and inhibit haemocytes accumulation so can become anti-inflammatory substance.

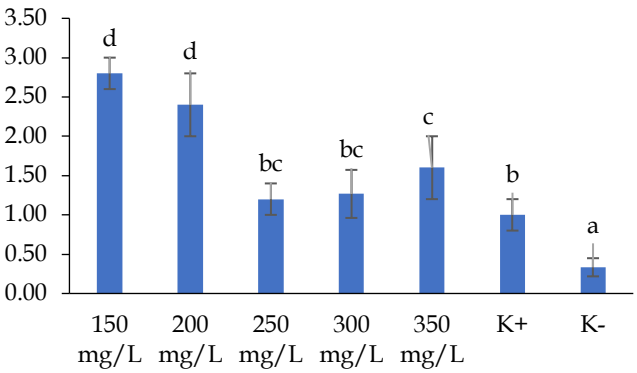


Figure 3. Vannamei Hepatopancreas Hemorrhage Scoring Results

Hepatopancreas histology in 300 and 350 mg/L doses experienced enhancement hemorrhage damage value. This increase suspected because the dose has exceed from optimal dose, so the leaf extract is not influence and possibility can be toxic for shrimp. The dosage of flavonoids that too high precisely can become toxic and even can cause death (Musdalipah et al., 2021). Based on one-way anova test in Table 6, obtained results

that necrosis damage to the hepatopancreas tissue show significant result ($P < 0.05$).

Research results show that purik leaf extract treatment can lower hepatopancreas necrosis in vibrio infected vannamei. The lowest necrosis damage is in 250 mg/L (C) treatment dose with damage value by 1.20%. Necrosis is cells death or damaged with very low activity off cells. In general this damage cannot be cured or irreversible. According to Miller & Zachary (2017), this characteristic of damage is irreversible that cannot be recovered. Flavonoid content in the purik leaf extract at the correct dosage capable to prevent enzymatic disturbance. Ullah et al. (2020) stated that flavonoids are capable to be an inhibitor of radical free formation enzyme. Necrosis is interaction results between radical free from drug metabolism and body metabolism with biomolecules of the hepatopancreas cell membrane.

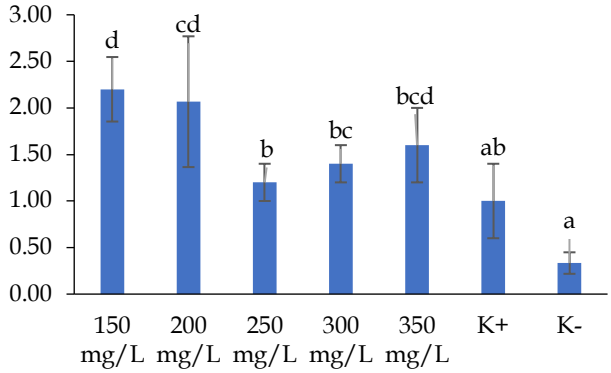


Figure 4. Vannamei Hepatopancreas Necrosis Scoring Results

Based on one-way anova test in Table 6. obtained results that cell inflammation to the hepatopancreas tissue show significant result ($P < 0.05$).

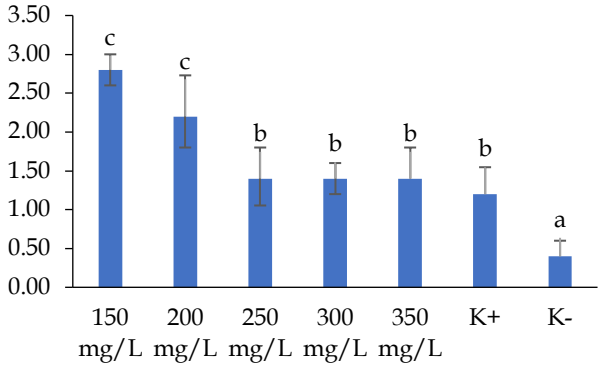


Figure 5. Vannamei Hepatopancreas Necrosis Scoring Results

Cell inflammation are the part of the immune system. Inflammation is important body mechanism for defending against various hazards or infection and also restore the cell structure (Akrom et al., 2022). The lowest cell inflammation value is dose C treatment with 250 mg/L extract and has a close value to K+ and K-, so treatment C is best treatment dose at this research. Flavonoid and alkaloid compounds in purik leaves as antioxidants function neutralize radical free as well as minimize the damage effect to cells and body tissues. Flavonoids and alkaloids as anti-inflammatory capable regenerate cell damaged cells (Tandi et al., 2020).

Conclusion

Based on the results of a study on the use of purik leaf extract (M. speciosa), it can be concluded that purik leaf extract at a dose of 250 mg/L is the best dose for treating vaname shrimp infected with vibriosis by restoring hemocyte components and reducing hepatopanceras damage.

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Author Contributions
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Conflicts of Interest
The authors declare no conflicts of interest.

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