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RESEARCH ARTICLE

Potency of Desert Rose (*Adenium obesum* (Forssk.) Roem. & Schult.) Flower Extract against Artificially Induced Furunculosis in Oranda Goldfish (*Carassius auratus auratus*)

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ABSTRACT

Oranda goldfish is an ornamental fish that developed worldwide. This industry faces serious problems such as *Aeromonas salmonicida* infection (furunculosis). Alternative therapy is required to be developed. One of them is using desert rose (*Adenium obesum* (Forssk.) Roem. & Schult.). This study aimed to elucidate the potency of desert rose flower extract (DRFE) against artificially induced furunculosis in oranda goldfish. The desert rose was extracted and its quercetin content was analyzed. Sixty oranda goldfish were used as a model. Fish were separated into I (healthy); II (infected); III (infected + 55 ppm ciprofloxacin); IV (infected + 1,000 ppm DRFE); V (infected + 2,000 ppm DRFE); VI (infected + 4,000 ppm DRFE). The therapy was given 5-days. On day six, the fish's blood and organs were collected. The samples were tested against haematology, histopathology, and immunohistochemistry. The data were analyzed by SPSS26. The results showed that DRFE contained quercetin (5.32±0.18%). The DRFE has potential benefits as healing promoters against furunculosis in oranda goldfish. It is indicated by significant differences in parameters: erythrocytes, haemoglobin, PCV, erythrocyte indices, leucocytes, differential leucocytes, histopathology, serology, and immune-expression of CD4+, CD8+, and ratio CD4+/CD8+ compared to the infected group ($P \leq 0.05$). The most significant concentration of DRFE was 4,000 ppm and it has no differences compared to 55 ppm ciprofloxacin ($P \geq 0.05$). The DRFE can be used as the alternative treatment against artificially induced furunculosis in oranda goldfish and it must be explored further against another type of bacterial infection.

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INTRODUCTION

Oranda goldfish (*Carassius auratus auratus*) is the one of ornamental fish that has been expanded in Indonesia and various countries. Oranda goldfish become the most famous fish among fanciers. A lot of local sellers import goldfish from overseas just to fulfil their demand in the Indonesian market. The import is conducted because of the limited production of local farmers. The limited production is caused by a hatching failure, growth disorder and metabolic and infectious diseases (Pękala-Safińska, 2018).

The infectious disease in oranda goldfish is commonly caused by *Aeromonas salmonicida* (Shameena *et al.*, 2021). The infection of this bacterium is known as furunculosis. Furunculosis impacts the oranda goldfish from the juvenile to the adult stage. It destroys the gills, liver, intestines, skin, kidneys, and brain. Furunculosis can be treated using antibiotics, including florfenicol. Bilen and Elbeshti (2019) reported that the efficacy of florfenicol against furunculosis reached 80%. Unfortunately, antibiotics utilization in aquaculture contributes to the occurrence of antimicrobial-resistant (AMR) (Schar *et al.*, 2021). So, it is necessary to develop alternative treatments against furunculosis and prevent the occurrence of AMR bacteria in aquaculture at once. One of them uses a herb-derived product, such as desert rose (*Adenium obesum* (Forssk) Roem. & Schult).

Desert rose contains quercetin. Quercetin is derived from bioflavonoids that have the potential as an antioxidant, anti-inflammatory, and antibiotic. The

previous study reflected that desert rose stem-bark extract inhibit the colonization of *S. aureus, P. aeruginosa, P. vulgaris* and *E. coli* during an in vitro study (Akhtar *et al.,* 2016). Lu *et al.,* (2021) elucidated that the antibacterial activity of desert rose stem extract is supported by its rosmarinic acid and quercetin content. Unfortunately, those previous studies have not analyzed the efficacy of desert rose flower extract (DRFE) against furunculosis in goldfish. Hence, this study aimed to analyze the potency of DRFE against artificially induced furunculosis in oranda goldfish.

MATERIALS AND METHODS

Ethic approval: The ethic committee from FVM, University of Wijaya Kusuma Surabaya has approved this study with number: KKE-67/XII/2021 in December 2021. The study was conducted from January until July 2022 in the Laboratory of Pharmacology, University of Wijaya Kusuma Surabaya.

Herbal preparation: The desert rose species was identified as *Adenium obesum* (Forssk.) Roem. & Schult. by the herbologist in Center for Research and Development of Medicinal Plants, Indonesia with number: KM.04.02/2/1247/2022. The desert rose flower was macerated using 96% alcohol and evaporated using an evaporator. The quercetin of DRFE was standardized using UV–Vis spectrophotometry (Pejic *et al.,* 2004). The result showed that DRFE contains 5.32±0.18 % quercetin.

Preliminary study: *A. salmonicida* isolate was cultured on the furunculosis agar. The growing colony was inoculated into peptone water. The inoculum turbidity was equalized with 0.5 McFarland standard. The inoculum was tested for minimum inhibitory concentration (MIC) against DRFE concentrations: 0, 0.25, 0.5, and multiples up-to 256 µL. The mixture was incubated and its turbidity was checked using spectrophotometer. Finally, 2 µL/mL became the MIC of DRFE and it was used in the next study.

Animal model and design: Sixty-oranda goldfish (*Carassius auratus auratus*) (weight: 22.13±4.45 g, length: 5.48±0.87 cm, 6-weeks-old) were used as a model. They were separated into six-groups. The group was: I (healthy); II (infected); III (infected + 55 ppm ciprofloxacin); IV (infected + 1,000 ppm DRFE); V (infected + 2,000 ppm DRFE); VI (infected + 4,000 ppm DRFE). The infection was conducted by intraperitoneal injection of 0.1 mL *A. salmonicida* inoculum. After 24-hours post-infection, the therapy was given using immersion for 1 hour/day for 5-days.

Sample collection: The behavioural changes and macroscopical lesions were recorded every day. The score of swim abnormality was: 1 = no swimming abnormalities; 2 = swimming oriented on one side; 3 = swimming upside down; 4 = cannot swim and stay on the base or the water surface. The score of appetite was, 1 = feed rate in ≤3 minutes; 2 = feed rate in >3 and ≤5 minutes; 3 = feed rate in >5 and ≤7 minutes; 4 = feed rate in >7 minutes. The score of skin colour was, 1 = no skin colour changes; 2 = redness in ≤30% of body; 3 = redness in >30% of the body. The score of skin lesion was, 1 = no skin lesions; 2 = increase mucus secretion; 3 = haemorrhage under the scale; 4 = haemorrhage and ulceration.

On day-6, the fish blood was collected via caudal vein. Further, the fish were euthanized and the organs were collected (i.e.: gills, liver, kidney, skin, and brain). The organs were fixed using 10% neutral buffer formalin.

Laboratory test: The haematology was measured using manual methods following described procedure by Witeska *et al.* (2022). C-reactive protein (CRP) was measured following the previous study (Kodama *et al.,* 2004). The organs were processed for routine histopathology using H&E staining. Cell tube block (CTB) was performed by inserted of blood into the plain capillary tube. The blood was centrifuged and its buffy coat was processed using immunohistochemistry against antibodies anti-CD4+ and anti-CD8+ (Prakoso *et al.,* 2020). The histopathology slide was scored using grading system for each parameter i.e.: 1 (no pathological changes); 2 (mild); 3 (moderate); 4 (severe). Further, the immunohistochemistry was analysed using Image-J and reported as percentage area.

Analysis data: The data were analysed using SPSS version 26 with a significance level of P≤0.05. The normal and homogeny data were then analysed using ANOVA. The inhomogenous and abnormal data were analysed using Kruskal-Wallis and Man Whitney-U test.

RESULTS

Behaviour and macroscopical lesions: In this study, the oranda goldfish showed significant behavioural changes and macroscopical lesions (Fig. 1a-d). The significant changes in behaviour and macroscopic lesion were identified from Group II (P≤0.05). The artificially induced furunculosis in Group II caused fish unable to swim, anorexia, and also impacts skin colour, haemorrhage and ulceration (P≤0.05) (Fig. 1b). Moreover, the treated groups using 55 ppm ciprofloxacin, 2,000 and 4,000 ppm DRFE potentially affect the improvement of swimming capability and decrease lesions that were similar to the healthy group (P≥0.05) (Fig. 1c-d). However, the appetite of treated groups is still lower than Group I (Table 1).

Haematology: *A. salmonicida* infection caused anaemia marked by decreased erythrocytes and haemoglobin in Group II. Contrarily, Group III and VI showed similar erythrocytes count compared to Group I (P≥0.05). Further, the haemoglobin in Group III, V, and VI showed a better increase than in Group II and III (P≤0.05). Group III and IV showed lower PCV levels than Group I, but higher than Group II (P≤0.05). Interestingly, the PCV level of Groups V and VI were higher than the others (P≤0.05). There is no significant difference regarding the platelets in Group III, IV, V and VI compared to Group I (P≥0.05) (Table 2).

The artificially induced furunculosis in oranda goldfish caused a significant increase in MCV and MCH compared to the others (P≤0.05). However, it has no impact on the MCHC (P≥0.05). The increase of MCV and MCH was also occurring in all treated groups. However, the elevate of MCV and MCHC in treated groups was not as high as in Group II (Table 2).

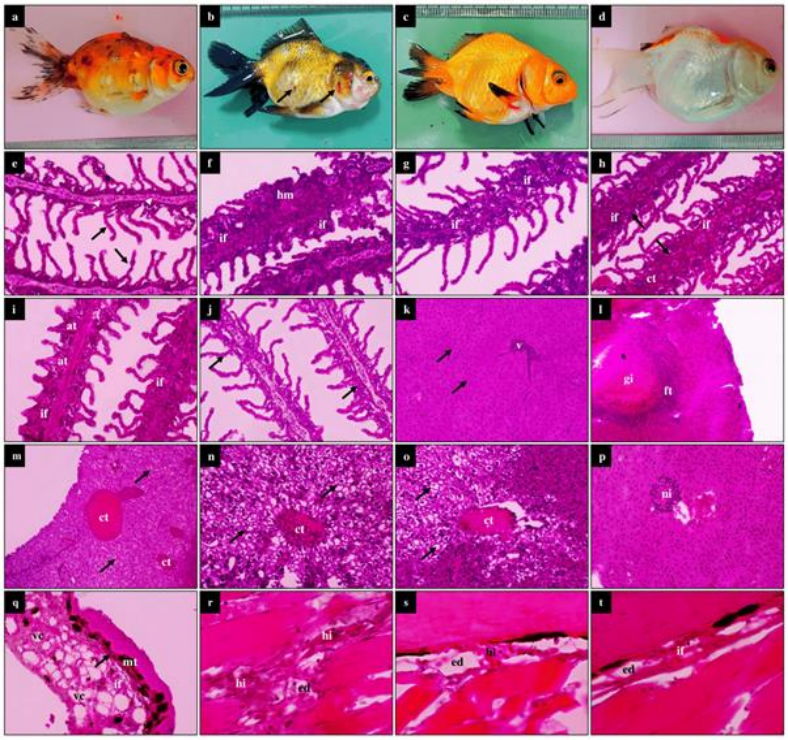

Fig. 1: Macrophotograph and histopathology of gills, liver, and skin from artificially induced furunculosis in oranda goldfish. Healthy oranda goldfish without lesions (a); ulcerative hemorrhage in operculum and abdomen (arrow) in fish from Group II (b); mild hemorrhage in Group II (arrow) (c); and normal appearance in Group VI (d); gill histology with thin lamellae (arrow) and filament (arrowhead) in Group I (e); severe epithelial hyperplasia (hm) and subepithelial leucocytes subpopulation (if) in Group II (f); subepithelial leucocyte subpopulation (if) within primary lamellae in Group III (g); the thickening of primary lamellae (arrow), congestion (ct) and leucocytes subpopulation (if) in Group IV (h); secondary lamellae atrophy (at) with subepithelial leucocytes subpopulation (if) in Group V (i); mild subepithelial leucocytes subpopulation (arrow) within primary lamellae in Group VI (j); liver histology with hepatocytes (arrow) and vein (v) in Group I (k); granulomatous inflammation (gi) surrounded by fibrotic tissue (ft) in Group II (l); congestion (ct) with vacuolated hepatocytes (arrow) in Group III (m); Group IV (n) and Group V (o); focal necrosis with lymphocytic infiltration (ni) from liver in Group VI (p); skin histopathology in Group II showed severe vacuolation (vc), lymphocytic inflammation (if) and increasing of melanophores (mt) on the basal layer (arrow) of epidermal part (q); severe heterophilic inflammation (hi) with muscle edema (ed) in Group II (r); moderate heterophilic inflammation (hi) with edema (ed) in Group III (s); and mild inflammation in Group VI (t). H&E, 100× (m, q), 400× (e-l, n-p, r-t).

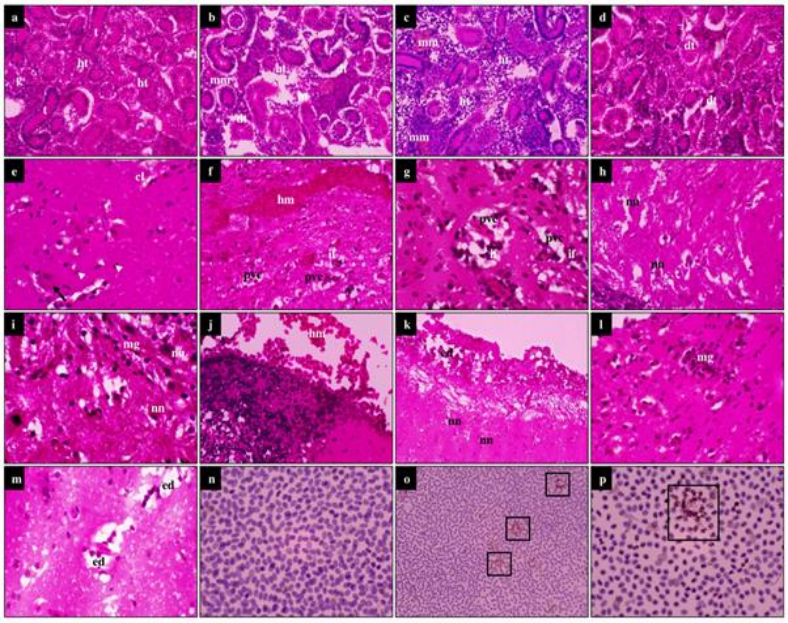

Fig. 2: Histopathology of kidney, brain, and immunohistochemistry of CD4+ and CD8+ from artificially induced furunculosis in oranda goldfish. Kidney histology with glomerulus (g), tubules (t), and hematopoietic tissue (ht) from fish in Group I (a); detached (dt) of renal tubules, loosening of hematopoietic tissue (ht), and increase of melano-macrophages (mm) in Group II (b); Group III (c); and mild detached (dt) of renal tubules in Group VI (d); brain histology with neuronal cell (arrow), neuroglial (arrowhead), and capillary (cl) from fish in Group I (e); Group II showed severe haemorrhage (hm) with lymphocytic (if) perivascular cuffing (pvc) (f, g); neuronal necrosis (nn) (h); necrosis neuronal (nn) with microgliosis (mg) in Group III (i); severe haemorrhage (hm) in Group IV (j); oedema (ed) and necrosis neuronal (nn) in Group V (k); mild microgliosis (mg) (l); and mild oedema (ed) around the blood vessel in Group VI (m); negative immune-expression of CTB from oranda goldfish (n); immune-expression of CD4+ marked by brown-colour (box) (o); and CD8+ (box) (p). H&E, 400× (a-f, h, j-k), 1000× (g, i, l-m); DAB, 1000× (n-p).

Leucocytes profile: There is an absolute increase of circulatory leucocytes, lymphocytes, monocytes, and heterophils in Group II compared to the others (P≤0.05). The lymphocytes and heterophils in Group III, IV, V, and VI are higher than in Group I, however, they showed a trend of decreasing compared to Group II (P≤0.05). In contrast, the treatment did not affect the monocytes (P≥0.05) (Table 2).

Histopathology: The artificially induced furunculosis in oranda goldfish has significant impacts on the score of histopathology in Group II, III, IV, V, and VI (P≤0.05). The histopathological changes were found in the gills, liver, skin, kidney and brain of all infected oranda goldfish either untreated or treated. Generally, 4,000 ppm DRFE has better effects on gills, liver, and skin histopathology compared to the 55 ppm ciprofloxacin group (p≤0.05) (Table 3). It is proved that there are no differences in those organs compared to the control (P≥0.05). Furthermore, ciprofloxacin has no significant differences regarding kidney and brain histopathology in all parameters compared to the 4,000 ppm DRFE group (P≥0.05) (Table 4). The ciprofloxacin and all concentrations of DRFE showed a better improvement in the prognosis of the infection marked by the decrease in histopathological changes compared to the untreated group (Fig. 1 and 2).

CRP, immune-expression of CD4+, CD8+, and ratio of CD4+/CD8+: There is an increase of CRP in Group II, IV, and V compared to the others (P≤0.05). It indicated that 1,000 and 2,000 ppm DRFE didn't show an effect on the CRP profile on artificially induced furunculosis in oranda goldfish. The significant decrease in CRP levels was indicated by Group III and VI (P≤0.05). A similar result was shown by

Table 1: Efficacy of ciprofloxacin and DRFE against behaviour and macroscopical lesions on artificially induced furunculosis in oranda goldfish.

Parameters	Group (mean±standard of deviation)					
	I	II	III	IV	V	VI
Swim abnormality	1.00±0.00[a]	3.80±0.63[b]	1.00±0.00[a]	1.50±0.97[c]	1.00±0.00[a]	1.00±0.00[a]
Appetite	1.00±0.00[a]	3.00±0.00[b]	2.00±0.00[c]	2.00±0.00[c]	2.00±0.00[c]	2.00±0.00[c]
Skin colour	1.00±0.00[a]	2.50±0.52[b]	1.40±0.51[a]	1.50±0.84[a, c]	1.00±0.00[a]	1.00±0.00[a]
Skin lesions	1.00±0.00[a]	3.50±0.70[b]	1.40±0.51[a]	1.50±0.84[a,c]	1.00±0.00[a]	1.00±0.00[a]

[a, b] = different superscript on the same row showed significant differences (P≤0.05).

Table 2: Efficacy of ciprofloxacin and DRFE against haematology and leucocyte profile on artificially induced furunculosis in oranda goldfish.

Parameters	Group (mean±standard of deviation)					
	I	II	III	IV	V	VI
Erythrocytes (× 10^6 sel/mm^3)	1.56±0.19[a]	0.96±0.09[b]	1.43±0.16[a]	1.38±0.10[c]	1.37±0.14[c]	1.42±0.19[a]
Haemoglobin (g/dL)	13.66±1.08[a]	10.71±0.85[b]	13.42±1.65[c]	11.15±1.57[b]	13.37±1.52[c]	13.08±1.55[c]
PCV (%)	28.26±3.96[a, d]	24.14±3.82[b]	27.79±2.29[c]	27.90±3.43[c]	29.47±2.78[c, d]	31.27±3.70[d]
Platelets (×10^3 sel/mm^3)	23.15±2.81[a]	36.36±7.46[b]	21.99±3.20[a]	26.48±4.30[a]	23.17±4.15[a]	20.81±1.34[a]
MCV (fL)	183.48±32.75[a]	254.62±50.85[b]	195.75±20.50[a]	202.92±28.89[a]	216.96±27.33[a, c]	224.90±46.46[c]
MCH (Pg)	88.88±13.29[a, c]	112.45±12.98[b]	95.14±17.48[c]	80.87±10.53[a]	105.95±16.15[c]	93.50±15.32[a, c]
MCHC (%)	49.16±7.49[a]	45.28±7.45[a]	48.36±5.27[a]	40.50±7.02[a]	49.42±9.23[a]	42.52±8.12[a]
Leucocytes (×10^3 sel/mm^3)	34.77±4.22[a]	54.60±11.20[b]	33.03±4.81[a]	39.76±6.45[a, c]	34.80±6.24[a]	31.25±2.02[a]
Lymphocytes (×10^3 sel/mm^3)	26.97±3.25[a]	37.36±7.56[b]	23.91±3.29[c]	28.29±4.53[a]	25.20±4.40[a, c]	24.31±1.75[c]
Monocytes (×10^3 sel/mm^3)	2.06±0.44[a]	3.83±1.46[b]	1.94±0.35[a]	2.44±0.61[a]	2.14±0.60[a]	1.90±0.31[a]
Heterophils (×10^3 sel/mm^3)	5.73±0.96[a]	13.40±4.00[b]	7.16±1.49[c]	9.02±1.62[c]	7.44±1.44[c]	5.02±0.66[a]

[a, b] = different superscript on the same row showed significant differences (P≤0.05).

Table 3: Efficacy of ciprofloxacin and DRFE against histopathology of gills, liver, and skin on artificially induced furunculosis in oranda goldfish.

Organ	Parameters	Group (mean±standard of deviation)					
		I	II	III	IV	V	VI
Gills	Epithelial hyperplasia	1.20±0.40[a]	3.40±0.66[b]	1.60±0.66[c]	2.70±0.64[d]	2.50±0.50[d]	1.70±0.64[c]
	Atrophy lamellae	1.20±0.40[a]	2.80±0.60[b]	1.80±0.40[c]	2.40±0.48[b]	2.20±0.60[c]	1.50±0.50[a]
	Inflammation	1.10±0.30[a]	3.30±0.64[b]	2.00±0.63[c]	2.50±0.50[d]	2.50±0.50[d]	1.40±0.48[a]
	Necrosis	1.10±0.30[a]	2.40±0.48[b]	1.40±0.48[c]	1.70±0.45[c]	1.50±0.50[c]	1.40±0.48[c]
	Congestion	1.40±0.66[a]	3.60±0.48[b]	1.90±0.53[c]	2.70±0.45[d]	2.30±0.64[d]	1.50±0.50[a]
Liver	Inflammation	1.30±0.45[a]	3.50±0.50[b]	2.30±0.64[c]	2.00±0.63[c]	1.70±0.45[d]	1.50±0.50[a]
	Necrosis	1.10±0.30[a]	2.60±0.48[b]	2.40±0.48[b]	1.90±0.53[c]	1.50±0.50[c]	1.30±0.45[a]
	Congestion	1.30±0.45[a]	2.40±0.48[b]	2.20±0.40[c]	2.00±0.63[c]	1.60±0.48[a]	1.50±0.50[a]
	Hepatocytes vacuolation	1.10±0.30[a]	3.40±0.48[b]	1.90±0.70[c]	1.70±0.45[c]	1.60±0.48[c]	1.60±0.48[c]
Skin	Inflammation	1.20±0.40[a]	3.30±0.78[b]	2.50±0.50[c]	3.00±0.63[c]	2.60±0.48[c]	1.60±0.48[d]
	Necrosis	1.10±0.30[a]	2.70±0.45[b]	2.30±0.78[c]	2.30±0.45[c]	2.30±0.64[c]	1.50±0.50[d]
	Vacuolation	1.00±0.00[a]	2.10±0.83[b]	2.50±0.50[c]	1.40±0.48[d]	1.60±0.48[d]	1.20±0.40[a]
	Increase of melanophores	1.10±0.30[a]	3.20±0.60[b]	2.60±0.48[c]	2.90±0.70[c]	2.30±0.45[c]	1.50±0.50[d]

[a, b] = different superscript on the same row showed significant differences (P≤0.05).

Table 4: Efficacy of ciprofloxacin and DRFE against histopathology of kidney and brain on artificially induced furunculosis in oranda goldfish.

Organ	Parameters	Group (mean±standard of deviation)					
		I	II	III	IV	V	VI
Kidney	Detached renal tubules	1.20±0.40[a]	2.50±0.50[b]	2.30±0.45[c]	2.70±0.64[b]	2.4±0.48[b]	2.20±0.60[c]
	Loosening hematopoietic tissue	1.30±0.45[a]	2.40±0.66[b]	2.30±0.64[c]	2.60±0.48[b]	2.40±0.66[b]	2,10±0.53[c]
	Increase melano-macrophages	1.40±0.66[a]	2.50±0.50[b]	2.30±0.45[c]	2.70±0.45[d]	2.20±0.60[c]	2.30±0.64[c]
Brain	Hemorrhage	1.20±0.40[a]	3.20±0.60[b]	2.70±0.64[c]	3.30±0.45[b]	3.20±0.40[b]	2.00±0.77[d]
	Congestion	1.20±0.40[a]	2.30±0.45[b]	1.90±0.53[c]	2.30±0.45[b]	2.20±0.40[b]	1.40±0.48[a]
	Microgliosis	1.30±0.45[a]	3.50±0.50[b]	2.10±0.70[c]	3.20±0.60[b]	2.50±0.50[c]	2.20±0.74[c]
	Inflammation	1.00±0.00[a]	3.50±0.50[b]	2.70±0.64[c]	3.30±0.45[b]	3.20±0.40[b]	2.40±0.48[c]
	Necrosis neuronal	1.20±0.40[a]	2.50±0.50[b]	1.90±0.53[c]	2.30±0.45[b]	2.20±0.40[b]	1.60±0.48[c]
	Edema	1.00±0.00[a]	3.30±0.45[b]	1.90±0.70[c]	2.80±0.40[d]	2.40±0.48[d]	1.50±0.50[e]
	Perivascular cuffing	1.00±0.00[a]	2.20±0.60[b]	1.10±0.30[a]	1.30±0.45[c]	1.20±0.40[a]	1.00±0.00[a]

[a, b] = different superscript on the same row showed significant differences (P≤0.05).

Table 5: Efficacy of ciprofloxacin and DRFE against CRP, immune-expression of CD4+, CD8+, and ratio CD4+/CD8+ on artificially induced furunculosis in oranda goldfish.

Parameters	Group (mean±standard of deviation)					
	I	II	III	IV	V	VI
CRP (mg/dL)	82.64±6.91[a]	105.26±16.22[b]	87.61±12.79[a]	102.84±14.75[b]	95.67±8.19[a, b]	84.00±7.25[a]
CD4+ (%)	4.00±0.80[a]	2.65±1.43[b]	3.96±0.96[a]	2.57±1.06[b]	3.17±0.49[b]	3.93±0.61[a]
CD8+ (%)	3.18±0.72[a]	4.75±1.52[b]	3.23±0.49[a]	2.60±0.82[a]	3.12±0.66[a]	2.95±0.51[a]
CD4+/CD8+	1.28±0.24[a]	0.56±0.26[b]	1.24±0.36[a]	1.02±0.42[c]	1.04±0.20[c]	1.37±0.33[a]

[a, b] = different superscript on the same row showed significant differences (P≤0.05).

the immune-expression of CD4+. The CD4+ in Group III and VI didn't different compared to Group I (P≥0.05). However, the CD4+ in Group II decreased significantly (P≤0.05). Finally, all the treated groups were significantly different regarding CD8+ and ratio CD4+/CD8+ compared to Group II (P≤0.05) (Table 5). The immune-expression of CD4+ and CD8+ was embedded in Fig. 2n-p.

DISCUSSION

Furunculosis is an infectious disease among the fisheries, including oranda goldfish farming. Furunculosis is caused by *A. salmonicida* infection. *A. salmonicida* transmit from the water to the internal organ of the fish. It has been proved by the previous study that the transmission

and colonization of the bacteria occur in the dorsal and pectoral fin and the gills, and they enter the internal organ via the opening of the fish anal (Bartkova *et al.,* 2017). *A. salmonicida* colonizes inside the fish's internal organ by producing the serine protease, adhesin, toxins, and phospholipase, utilizing myo-inositol as a sole carbon source (Azzam-Sayuti *et al.,* 2021), lipase, protease, and A-layer factor (Girard *et al.,* 2022).

Furunculosis causes mortality with severe histopathological findings. *A. salmonicida* infection triggers septicemia, branchitis, enteritis, fin rot, dermatitis, and death. Another study reported that the aeromonad infection causes necrosis of gills, hemocyte aggregation, gastrointestinal disorders, and enteritis (AlYahya *et al.,* 2018). It is related to this study that furunculosis impacts branchitis, dermatitis, myositis, nephritis, granulomatous hepatitis, and hemorrhagic encephalitis after 5-days of infection. Those histopathological changes in the untreated group seriously impact the changes in blood rheology i.e.: macrocytic hypochromic anaemia, thrombocytosis, leukocytosis (lymphocytosis, monocytosis, and heterophilia), elevated of CRP and decrease ratio CD4+/CD8+. The pathogenesis of furunculosis in fish influences the destruction of erythrocytes and haemorrhage that simply causes anaemia and thrombocytosis. The massive erythrocyte destruction impacts the decrease of haemoglobin and MCHC (Kondera *et al.,* 2021). Further, the circulating bacteria promotes leukocytosis. In severe cases, they penetrate the hematopoietic organs and stimulate hematopoietic disorders (Ghiasi *et al.,* 2016).

Furunculosis causes swim abnormalities due to the inflammation of the respiratory system. The inflammation of the hepatopancreatic system causes a decrease in appetite. One of the pathognomonic lesions of the *A. salmonicida* infection is a skin ulcer. The skin ulcer is caused by inflammation and rupture of muscle and dermal part of fish skin that changes skin colour.

The treatment of furunculosis is commonly used antibiotics, including ciprofloxacin. Ciprofloxacin is the potential in inhibiting the DNA replication of bacteria, and it has been reported as susceptible to *A. salmonicida* (Tewari *et al.,* 2014)). This study showed that the utilization of ciprofloxacin in oranda goldfish improves the haematological profile, leukocytes, monocytes, CRP, and the ratio of CD4+/CD8+ compared to the healthy group. It indicates that ciprofloxacin can penetrate through the fish body and its circulatory system via immersion. However, ciprofloxacin negatively affects the number of circulatory lymphocytes and heterophils. The high number of circulatory lymphocytes following the administration of ciprofloxacin because the lymphocytes are still actively conducting the healing of infected tissue by modulating the CD4+/CD8+ (Prakoso and Wijayanti, 2022). The immune-expression of CD4+ has an essential role in releasing cytokines against invasive pathogens in teleost fish (Ashfaq *et al.,* 2019) and CD8+ is essential in initiating of immune response (Jung *et al.,* 2021). While the toxin-related product of *A. salmonicida* triggers heterophilia. Those hypotheses are supported by the finding of inflammation that predominantly infiltrated by heterophils and lymphocytes in observed organs after ciprofloxacin therapy.

The immersion using 4,000 ppm DRFE showed better results on the haematological profile compared to the untreated group which is indicated by the haematology, serology, immune-expression of CD4+, CD8+, and ratio CD4+/CD8+. This group was not different compared to the healthy group. However, the histopathology finding is similar to the ciprofloxacin group. The potency of DRFE against artificially induced furunculosis due to its quercetin content (Ullah *et al.,* 2020).

Quercetin is widely present in plants-derived products (David *et al.,* 2016). It is commonly used as an antioxidant with several mechanisms: scavenging activity in ROS, chelating metal, inhibiting lipid peroxidation, regulating glutathione levels and promoting signal transduction relating to gene antioxidant expression (Yang *et al.,* 2020). Furthermore, quercetin has a broad-spectrum antibiotic mechanism. Wang *et al.,* (2018) described that quercetin potentially inhibits the growth of *S. aureus, P. aeruginosa, Listeria* sp., and *Shigella* sp. with MIC at ±2.00 mg/mL. Nguyen and Bhattacharya (2022) described that quercetin damaged the membrane wall of *S. aureus* and *E. coli* that leading to cavitation and bacterial cell death. Quercetin can also act as an antibacterial agent due to its activities during inhibiting DNA synthesis, decreasing the dry weight of bacterial biofilm, alteration of blaVIM and ompC expression (Kashiwagi *et al.,* 2021). It is similar to this study that quercetin from DRFE potentially increases the healing of oranda goldfish after artificially induced furunculosis. The mechanism of repair after treatment in this study can be observed grossly. The repair of the hematopoietic and hepatopancreatic system promote the appetite. Further, the ciprofloxacin and DRFE groups decrease brain inflammation which increases the swim abilities of infected oranda goldfish. The major lesion that becomes the first screening for furunculosis is skin ulceration. This study proved that skin lesions of an infected group can be decreased by ciprofloxacin and DRFE.

Conclusions: The DRFE can be used as an alternative treatment against furunculosis in oranda goldfish. The effective concentration of DRFE that shows similar effects with ciprofloxacin is 4,000 ppm. Quercetin supports the efficacy of DRFE against furunculosis. The potency of DRFE must be explored further using another type of eminent bacteria in aquaculture.

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