Egyptian Journal of Aquatic Biology & Fisheries
Zoology Department, Faculty of Science,
Ain Shams University, Cairo, Egypt.
ISSN 1110 – 6131
Vol. 29(6): 1687 – 1712 (2025)
www.ejabf.journals.ekb.eg

Ulva alga Mitigates *Aeromonas hydrophila* Infection in *Oreochromis niloticus*: Pathological and Chemical Assessment

Naglaa M. Alkalamawey[1], Ahmed M. Elmahdy[2], Hend Fouad[2], Ahmed M. Heneash[3], Jehan I. Abdellatief[4]

[1]Molecular Pathology Unit-Pathology Dept., Animal Health Research Institute, ARC, 12618, Egypt
[2]Department of Biochemistry, Pharmacology, Toxicology and Feed Deficiency, Animal Health Research Institute, ARC, 12618, Egypt
[3]Hydrobiology Department, National Institute of Oceanography, and Fisheries, Egypt
[4]Fish Diseases Research Department, Animal Health Research Institute, Dokki, ARC, 12618, Egypt
[*]**Corresponding Author: jjibrahim2001@gmail.com**

ARTICLE INFO

Article History:
Received: Aug. 20, 2025
Accepted: Nov. 8, 2025
Online: Dec. 4, 2025

Keywords:
O.niloticus,
Aeromonas hydrophila.
Antibacterial,
Ulva alga,
Histopathological changes

ABSTRACT

Misuse of antibiotics in fish aquaculture causes antimicrobial resistance (AMR), which lowers the efficacy of antibiotic treatment. The prevalence of antibiotic residues in animal products poses a concern to the general public's health. An additional concern is the horizontal transfer of resistance determinants from aquatic bacteria to the human pathogen. This study aims to combat multidrug resistance by including compounds with antimicrobial activity and low risk of developing antimicrobial resistance. *Ulva* algae were collected from the Egyptian aquatic environment (Mediterranean Sea) and subjected to chemical analysis of major components. The antibacterial activity of extracted fatty acids and polysaccharides was evaluated *in vitro*. The results revealed that the whole fatty acid extract exhibited antibacterial effects, primarily against Gram-negative bacterial strains (*Aeromonas hydrophila*, *Shewanella* spp., *Proteus vulgaris*, and *Escherichia coli*). However, no antibacterial activity was observed against the tested Gram-positive strains (*Enterococcus faecalis* and *Staphylococcus aureus)*. In contrast, the extracted polysaccharides showed no detectable antibacterial activity against any of the tested bacterial strains. A follow-up *in vivo* investigation was carried out with 120 healthy *Oreochromis niloticus* (70 ± 5g) split up into four groups (n=30 each). For four weeks, fish in the experimental group were given a diet enhanced with whole *Ulva* algae (1g/ kg feed). After the feeding period, *A. hydrophila* (0.1mL of 1×10^7 CFU/mL) was injected intraperitoneally to the challenge groups. The *Ulva*-supplemented groups exhibited a significantly lower mortality rate (30%) compared to the infected control group which recorded 80% mortality. Liver and kidney function markers, lipid profile, and tissue antioxidant parameters were assessed. Improved antioxidant status was indicated by biochemical analysis, which showed lower MDA levels and higher levels of CAT and GSH in the liver and kidney tissues. *Ulva*-treated groups showed improvements in hepatic and renal tissues as determined by histological analyses of the liver, kidney, and spleen.

INTRODUCTION

Antimicrobial resistance (AMR) phenomenon represents a great challenge for microbial disease treatment (**Karunasagar *et al.*, 1994**), which has prompted many governments to establish maximum residue levels (MRLs) for aquaculture products

(**FAO, 2002**). The WHO established the “One Health” approach aimed to tackle antimicrobial resistance (AMR) with the United Nations’ Sustainable Development Goals for 2030 for human, terrestrial, and aquatic animals, food and feed, plants and crops (**WHO, 2020**).

The marine environment is seen as a source of new bioactive compounds and represents a high ecological richness (**Srinivasan *et al.*, 2021**). As photosynthetic creatures, macro-algae may help maintain the maritime environment. They contain a wide range of bioactive substances, including proteins, lipids, vitamins, minerals, polysaccharides, macrolides, polyphenols, and fatty acids (**Manivasagan *et al.*, 2014**).

Antimicrobial substances derived from marine organisms and medicinal plants, such as phenolics, steroidal glycosids (**Hemaiswarya *et al.*, 2008**), carbohydrates (**Pasdaran & Hamedi, 2017**), terpenoids, alkaloids, sulfur-containing compounds, and fatty acids (**Yoon *et al.*, 2018; Khameneh *et al.*, 2019**), with the advantages of other primary and secondary metabolites, are acknowledged as "generally recognized as safe" (GRAS), meaning that there is little possibility of AMR triggering (**Stefanović, 2018**).

Natural products that have therapeutic or protective antibacterial effects generally have a non-specific mode of action; they can improve fish gut health and nutrient absorption by promoting the growth of beneficial bacteria and/or inhibiting the proliferation of harmful bacteria, improving the innate immune response, modifying the expression of cytokines, enhancing antioxidant capacity, and/or increasing disease resistance (**Siddik *et al.*, 2023**). As a result, natural items are utilized as feed additives instead of pharmaceuticals.

Dietary supplementation with yeast culture (**Bu *et al.*, 2019**) and *Lagnaria breviflora* leaves extract enhances immunological response and antioxidant ability to strengthen catfish's resistance to *A. hydrophila* infection (**Hou *et al.*, 2022; Paray *et al.*, 2024**). Similar effects were recorded in juvenile gibel carp and the Nile tilapia (**Abdel-Wahab *et al.*, 2021**) against *A. hydrophila* infection.

Phlorotannins, fatty acids, polysaccharides, peptides, terpenes, polyacetylenes, sterols, indole alkaloids, aromatic organic acids, shikimic acid, polyketides, hydroquinones, alcohols, aldehydes, ketones, halogenated furanones, alkanes, and alkenes are among the secondary metabolites of marine algae with varying pigmentations (red, brown, and green). The mode of action of some of these compounds is still being studied (**Shannon & Abu-Ghannam, 2016)**.

Both biotic and abiotic environmental factors contribute to the large range of chemical compositions found in the secondary metabolites of algae. Therefore, the defensive mechanism of the algae against its sheltering polluted environment and its endeavor to

protect its persistence were credited with the antibacterial function of certain marine macro algae (**Ismail *et al*., 2018**).

Ulva algae crude extract revealed a stronger anti-bacterial effect than standard streptomycin against MRSA **(Gerstel *et al*., 2018)**.

An opportunistic pathogen rod-shaped and Gram-negative is *A. hydrophila*. It is commonly found in aquatic habitats and can cause serious illnesses in fish, including septicemia, ulcerative disorders, and fin rot **(Janda & Abbott, 2010; Sherif & Kassab, 2023)**. Multiple antibiotic resistance in *A. hydrophila* has made treating fish infections more difficult. Numerous studies reported high levels of resistance to common antibiotics, including ampicillin, tetracycline, and chloramphenicol **(Ayoub *et al*., 2024; Sitteen *et al.*, 2024)**. Infections caused by *A. hydrophila* in the Nile tilapia can result in high mortality rates, which can impact aquaculture operations' economic viability and productivity **(Anantasuk *et al*., 2024)**.

The study distinguishes that *Ulva* alga (green algae) could be safely added to the diet feed for fish, and this provides *O. niloticus* with strong protection against *A. hydrophila* infection.

MATERIALS AND METHODS

Biosafety measures*:* This study followed the biosafety measures according to pathogen safety data sheets: Infectious substances *Aeromonas hydrophila,* Pathogen Regulation Directorate **(Public Health Agency of Canada, 2019)**.

1. *Ulva* algae*:* Algae were collected from the Mediterranean Sea beaches in Alexandria Governorate.

2. Chemical analysis

Chemical analysis of the major components of *Ulva* algae revealed the following: ash content **(AOAC 962.09, 2015)**, protein content **(AOAC 930.15, 2015)**, carbohydrate content (calculated mathematically), crude fiber **(AOAC 2003.05, 2015)**, moisture content **(AOAC 942.05, 2015)**, and fat content **(AOAC 2001.11, 2015)**.

2.1. Total fatty acids extraction

Dried Ulva algae were ground, and fatty acids were collectively extracted according to **Jiang *et al*. (2013)**.

2.2. Polysaccharide extraction

Water-soluble polysaccharides were extracted from *Ulva* algae following the method described by **Phomkaivon *et al.* (2024)**. Deionized water was added to the algae biomass, which was then incubated in a water bath with hand stirring every ten minutes. To extract the soluble polysaccharides, the mixture was centrifuged for 20 minutes at 3660×g following the extraction period. The water-soluble polysaccharides that were gathered were further examined.

3. Anti-bacterial effect of *Ulva* algae against bacterial pathogens

3.1. In vitro investigation

100ml of TSA media plates and broth of various bacterial strains (0.5 Standard MaFarland) contained 0.1, 0.5, and 1 ml of fatty acids, polysaccharide extracts, and crude algae separately. These strains included Gram-positive (*Enterococcus faecalis, Staphylococcus aureus*) and Gram-negative (*E. coli, Proteus vulgaris, Shawnella, A. veronii,* and *A. hydrophila*) bacteria. The broth and plates were incubated for 24 hours at 25°C.

3.2. In vivo investigation

3.2.1. Experimental design:

A total of 130 apparently healthy *O. niloticus* (70 ± 5g b.w.) were acquired from a private fish farm in Elfayom Governorate that had no disease records. The fish were then moved with an oxygen supply to the Fish Disease Department, wet lab unit, AHRI (Dokki). Throughout the experiment, the water's physicochemical characteristics stayed within the ideal range. A commercial meal comprising 30–35% crude protein per day at 3% body weight was fed to the fish, according to the method implemented by **Eurell *et al.* (1978)**.

The fish meal (in pellet form) was carefully soaked and thoroughly mixed until a paste was created. The consistency of the feed was then improved by adding the gelatin (Canal AquaCure, Egypt) to the feed paste/Ulva algae mixture. After allowing this mixture to dry at room temperature, it was then cut into uniformly small pieces.

Ten randomly chosen fish were subjected to a bacteriological investigation following two weeks of acclimation to guarantee pathogen-free conditions. Each of the four groups of fish had three replicates:

G1 (Control normal): *O. niloticus* fed on *Ulva* algea diet free (commercial diet 0.3% of fish body weight) 3 times daily.

G2 (Control challenged): *O. niloticus* fed on commercial diet for 4 weeks before challenged with I/P injection by a dose of 0.1ml ($1X10^7$ cfu) (**Brooks, *et al.*, 20215**) *A.hydrophila* strain (obtained from AHRI and submitted to the GenBank database under accession no. OL771444).

G3 (Algae control): *O. niloticus* fed on a diet containing *Ulva* algea at a concentration of 1g/ kg.

G4 (Algae/challenged group): *O. niloticus* fed on a diet containing *Ulva* algea at a concentration of 1g/kg for 4 weeks, followed by challenge with I/P injection by dose 0.1ml ($1X10^7$ cfu) *A. hydrophila.* A week after the challenge, clinical symptoms and morbidities were documented **(Schaperclaus, 1992)**. *A. hydrophila* was confirmed to be present in the isolates using bacterial re-isolation from challenged groups using Diagnostics SRO GN24 (www.diagnostics.sk), following the manner described by **Austin and Austin (2016)**

The number of dead fish was recorded, and the mortality rate during a specific period (MR) was measured using the following equation: MR (%) = number of deaths ⁄ total fish number × 100

3.2.2. Biochemical analysis:

Fish were anesthetized within 60s using 50mg/ L MS222(tricaine methanesulfonate). Blood samples were collected from all groups at the end of the experimental period to get sera.

3.2.2.1. Serum parameters:

Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were measured according to the method described by **Reitman and Frankel (1975)** and **Young (1997**), total protein and albumin were measured according to **Tietz (1994**), and by deducting the amount of albumin from the amount of total proteins, the mathematical level of globulin was determined. Serum urea and creatinine levels were quantitatively estimated according to **Wybenga (1971)** and **Tietz (1986)**, respectively.

3.2.2.2. Liver and kidney tissues:

Reduced glutathione (GSH) level was determined by a method described by **Beutler *et al.* (1963)**; malon-di-aldehyde (MDA) was estimated by **Satoh (1978)** method, and catalase (CAT) was measured according to **Aebi (1984)**. Total cholesterol (TC) was estimated according to **Watson (1960)**; triglyceride (TG) was measured by **Wahlefeld (1974)**; high-density lipoprotein (HDL) was evaluated according to **Peace and Kaplan (1987)**; and low-density lipoprotein (LDL) was evaluated in accordance with the study of **Young (1995)**.

3.3. Histopathological examination

Samples were taken from the kidneys, spleen, and liver at the conclusion of the experiment. Sections of formalin-fixed paraffin embedded were regularly prepared for H&E staining using the techniques outlined by **Suvarna *et al.* (2012)**.

4. Statistical analysis

The data were presented as mean + SE. One-way ANOVA and Tukey's post- *hoc* test for multiple group comparisons were used to examine the data using the statistical software program SPSS for Windows (version 21.0; SPSS Inc., Chicago, IL, USA). $P \leq 0.05$ was used to determine that the differences were statistically significant.

RESULTS

1. Chemical analysis of *Ulva* alga

Ulva alga employed in this experiment had the following chemical composition: 52% ash, 13.4% protein, 13% carbohydrate, 12% crude fiber, 6% moisture, and 2.7% fat.

2. Anti-bacterial effect of *Ulva* algae against bacterial pathogens

2.1 In vitro

Gram-negative bacterial growth was shown to be inhibited by the extraction of 1.0ml fatty acids and 0.1g of crude algae (Figs. 1, 2), with antibacterial activity against *A. hydrophila* in comparison to crude algae. Polysaccharides extraction did not exhibit an anti-bacterial effect against any of the investigated bacterial pathogens.

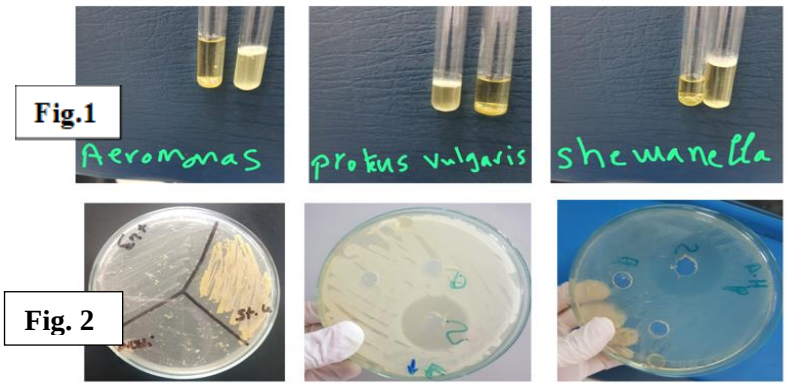

Fig. 1. Antibacterial effect of *Ulva* algae fatty acids extraction or crude algae on some Gram-negative bacterial broth

Fig. 2. Antibacterial effect of *Ulva* algae detected on *Aeromona*s spp, meanwhile no antibacterial effect was detected on Gram-positive strains on TSA media

2.2. In vivo

Following challenge with *A. hydrophila* (G2), morphological lesions of *O. niloticus* groups revealed significant congestion, bleeding at the operculum, and widespread skin ulceration with hemorrhagic boundaries (Fig. 3a). All internal organs were shown to be congested in the challenged group (G2) postmortem (Fig. 3b). Algae-fed/challenged group (G4) showed slight body color darkness, some hemorrhages over the head (Fig. 3c), and congestion of internal organs (Fig. 3d)

.

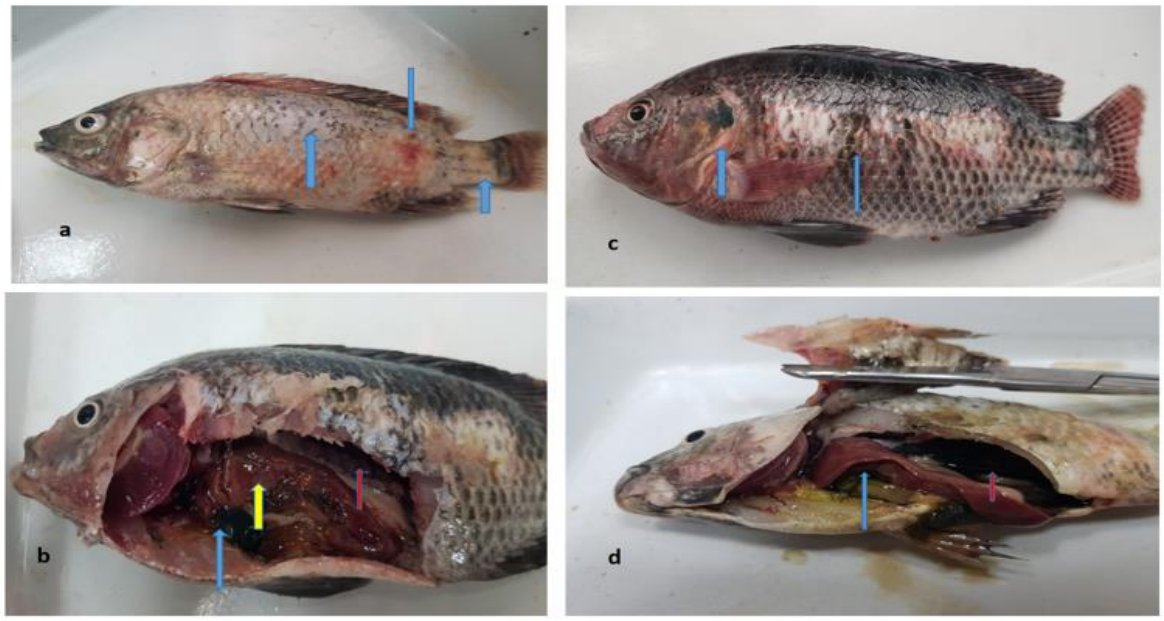

Fig. 3. *O. niloticus* experimentally injected with *A.hydrophila* (G2); showing hemorrhagic ulcer with loss of scales all over the body (a); darkness spleen(blue arrow) , congested liver (yellow arrow) and severely congested kidney (red arrow) (b)

G4(Algae fed/challenged group) showing, slight body darkness (c); congestion of liver (blue arrow) and kidney (red arrow) (d).

The morbidity rate began on the third day after the *A. hydrophila* challenge infection; in G2, it was 25/30-83.3 percent, and in G4, it was estimated to be 14/30-46.6 percent, and it manifested one week after the challenge. In G4, the mortality rate was 9/30-30%, whereas in G2, it was 24/30-80%.

Using the standard biochemical and diagnostic kit SRO GN24, *A. hydrophila* was re-isolated from the liver and kidney of morbidity and recently deceased fish (G2 and G4). The results showed that the fish were G-ve, oxidase positive, motile short rods, using citrate, hydrolyzing urea, and fermenting glucose, sucrose, and mannitol—all of which are characteristics of *A. hydrophila*.

3. Chemical parameters

While there were no discernible variations in urea and creatinine levels across all experimental groups, *A. hydrophila* infection markedly raised AST and ALT serum levels in G2 when compared to groups 1, 3, and 4 (Fig. 4). Significantly higher levels of LDL cholesterol, triglycerides, and cholesterol were seen in G2, while HDL and cholesterol levels were significantly lower in G2 compared to groups 1, 3, and 4 (Fig. 5). Significantly more globulin and lower albumin were detected in G2 compared to groups 1, 3, and 4. Total protein levels in each of the experimental groups did not differ significantly (Fig. 6). Comparing G2 to groups 1, 3, and 4, there was a large increase in MDA and a significant drop in GSH and CAT (Fig. 7). Significantly higher MDA and significantly lower GSH and CAT were recorded in G2 compared to groups 1, 3, and 4 (Fig. 8).

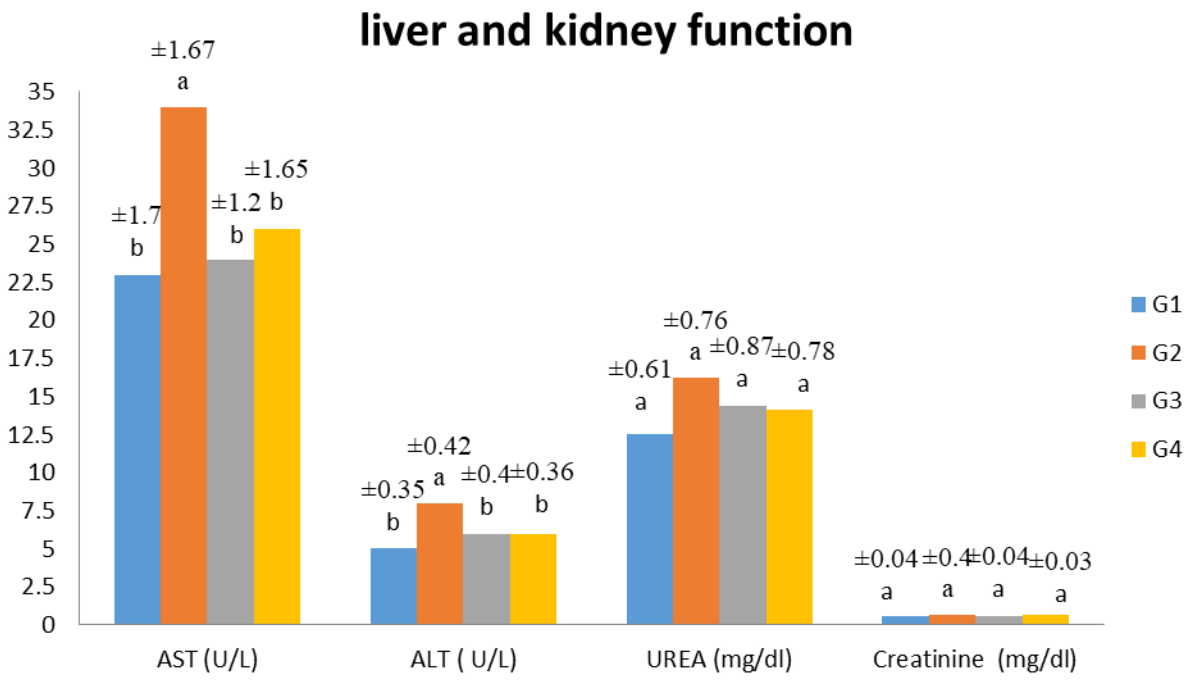

Fig. 4. Experimental impact of *Ulva* algae and/or *A. hydrophila* on AST, ALT, urea, and Creatinine in serum of *O.niloticus* groups
(n=3)

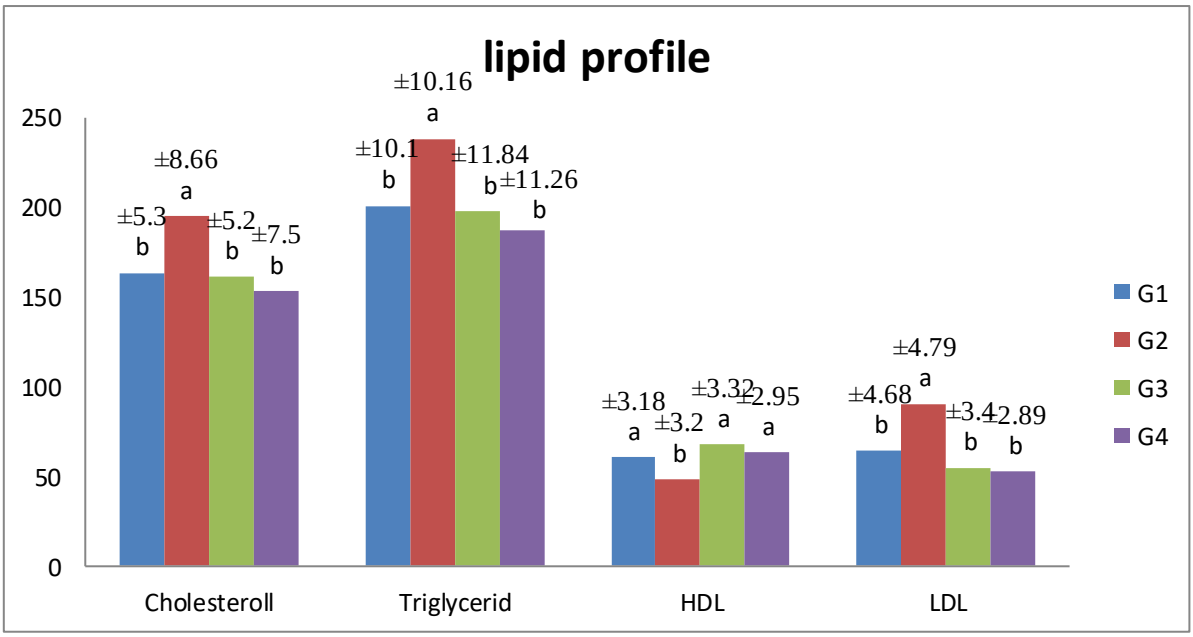

Fig. 5. Experimental impact of *Ulva* algae and/or *A. hydrophila* on cholesterol, triglyceride, HDL and LDL in serum (unit mg/dl)(of *O.niloticus* groups (n=3)

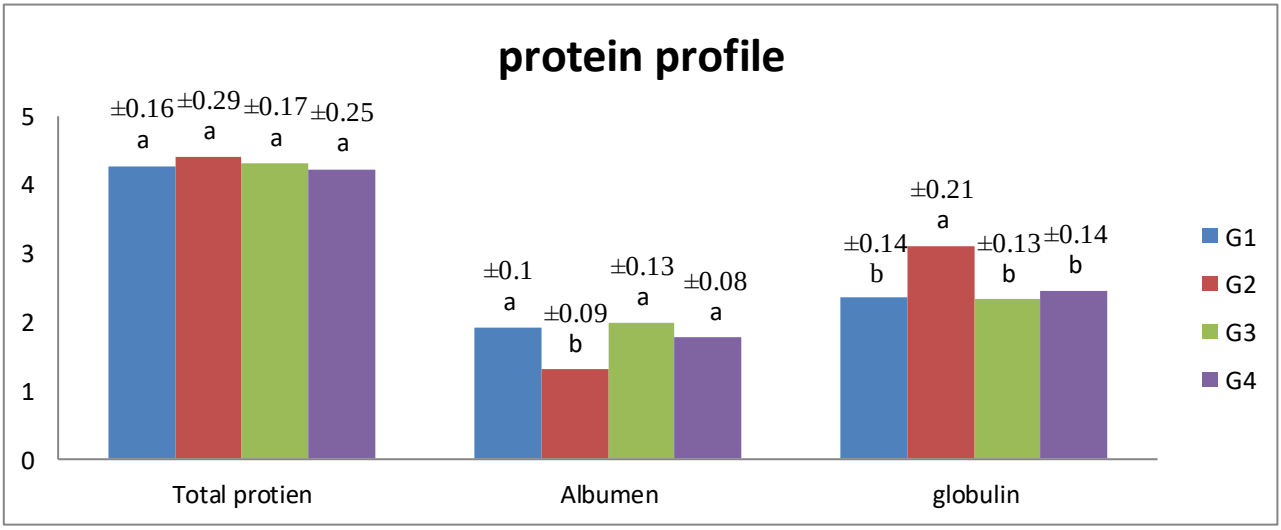

Fig. 6. Experimental impact of *Ulva* algae and/or *A. hydrophila* on total protein, albumin, and globulins in serum(unit mg/dl) of *O.niloticus* groups (n=3)

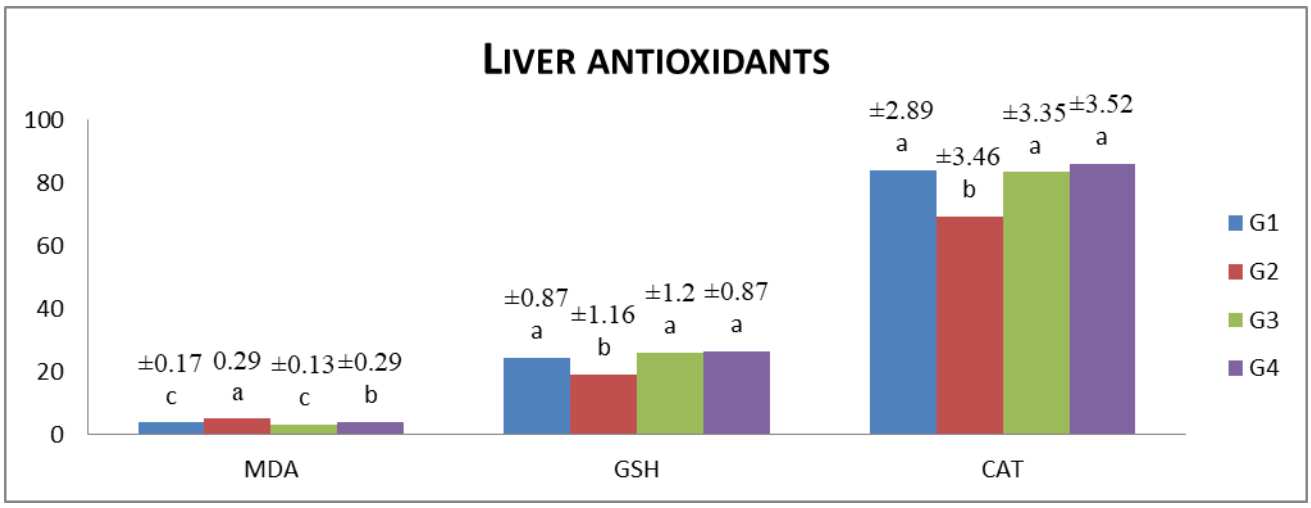

Fig. 7. Experimental impact of *Ulva* algae and/or *A. hydrophila* on MDA(nmol/g), GSH(nmol/g), and CAT(µg/g) in the liver of *O.niloticus* groups (n=3)

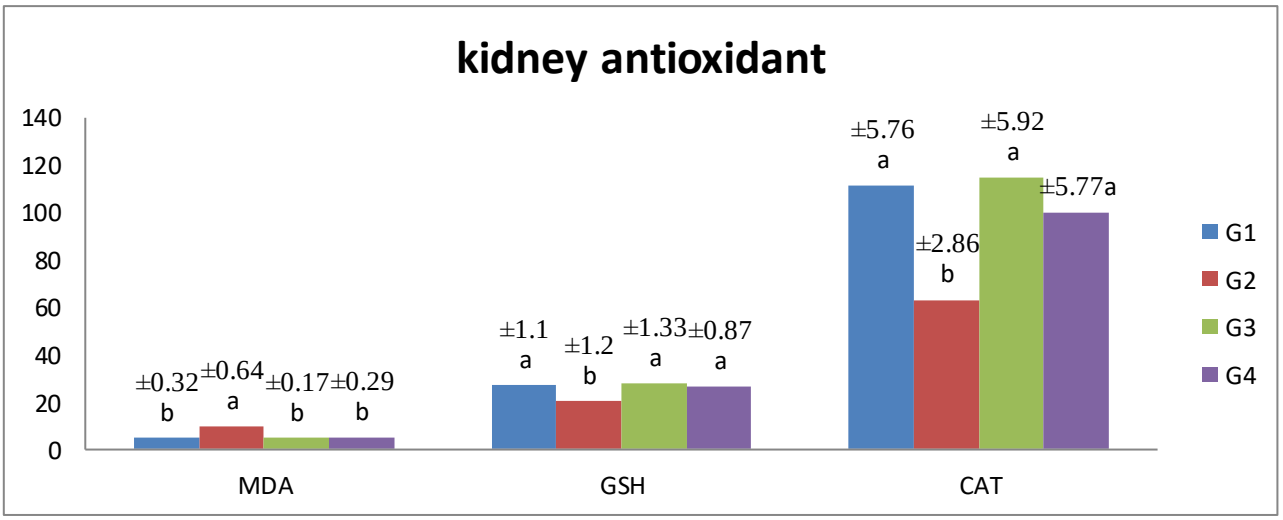

Fig. 8. Experimental impact of *Ulva* algae and/or *A. hydrophila* on MDA(nmol/g), GSH(nmol/g), and CAT(µg/g) in kidneys of *O.niloticus* groups (n=3)

.

4. Histopathological investigation

In G1 and G3, we found normal histological architectures in the kidneys, liver, and spleen. The G4 group had normal renal histology but noticeable hepatic sinusoidal congestion and splenic parenchymal destruction. In addition to hepato-pancreatic exocrine cell necrosis, G2 showed significant hydropic degeneration of hepatocytes with foci of necrosis linked to inflammatory cell infiltration. There have been reports of

Bowman's gap dilatation and significant deterioration of the tubular lining epithelia. Melano-macrophage centers and lymphocytic concentration were significantly reduced in the spleen. G3's normal histological state may serve as a gauge of the food ration's safe dosage.

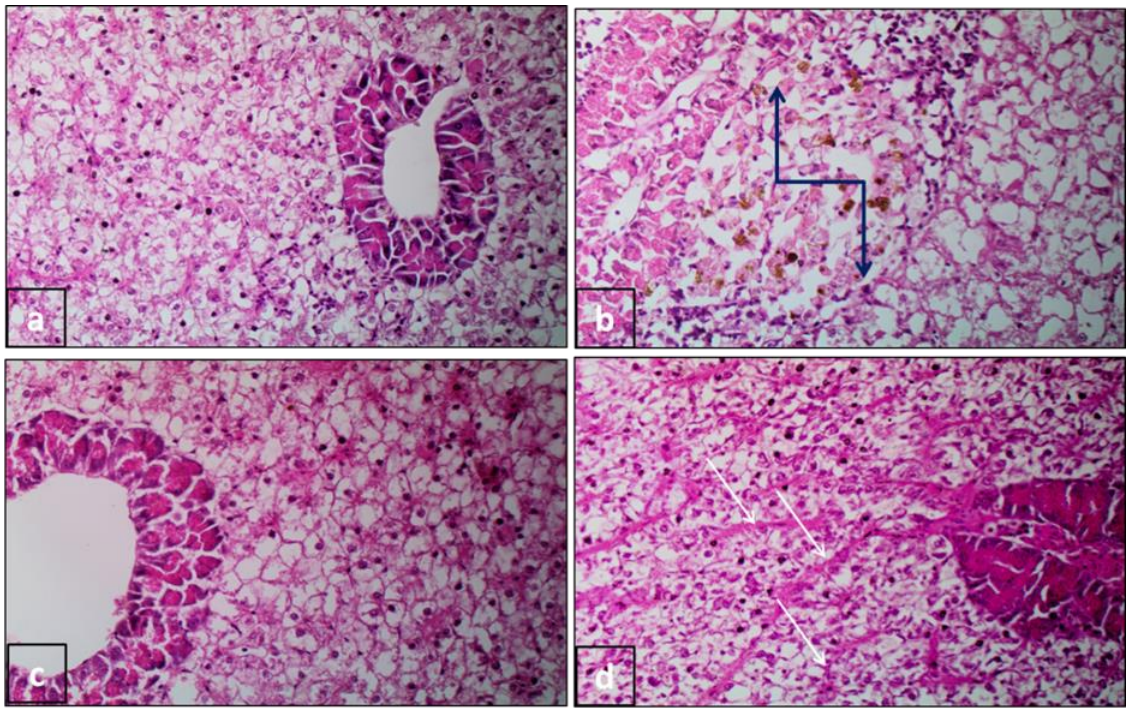

Fig. 9. Hepatic parenchyma reveal normal histological criteria in normal control (a) and algae feed (c) groups. *A.hydrophila* infected group revealed necrosis of hepato-pancreas , vacuolar degeneration of hepatocytes and foci of hepatocytes necrosis associated with inflammatory cells infiltration (truncated arrow) (b). Algae feed/challenged group showed marked congestion of hepatic sinusoids (thin arrows) (d).
H&E X 400

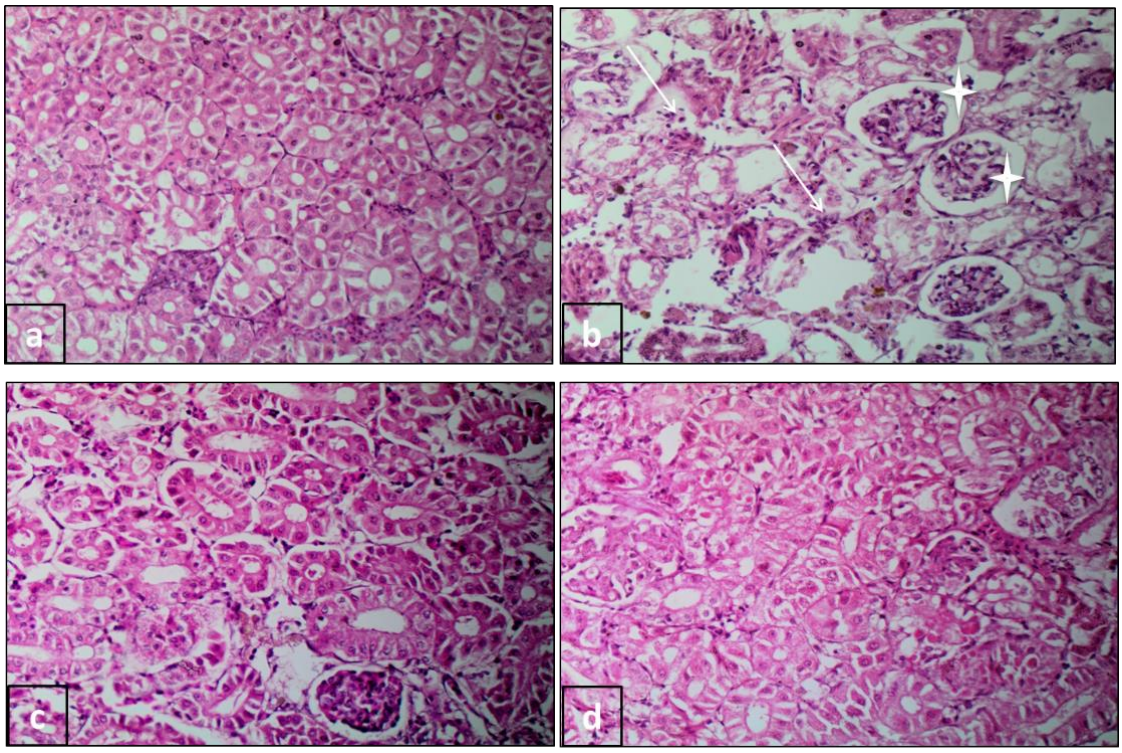

Fig. 10. Renal parenchyma reveal normal histological criteria in normal control (a), algae feed (c) and algae feed/challenged (d) groups. *A.hydrophila* infected (b) group showing hydropic degeneration of tubular lining epithelia with interstitial inflammatory cells infiltration (arrows) with marked dilation of bowman's space (asterisks). H&E X 400

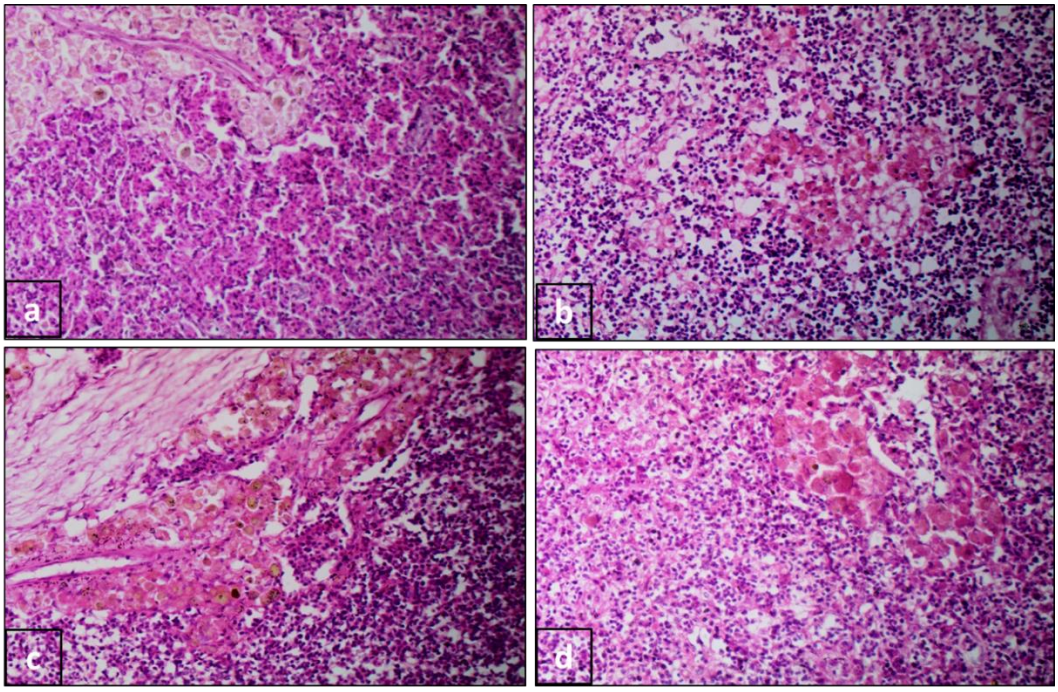

Fig. 11. Splenic parenchyma with red and white ellipsoids is detected in normal control (a), algae feed (c) and Algae- feed/challenged (d) groups. Depletion of lymphocytic content with deposition of fibrin and protein material is detected in *A.hydrophilla* infected (b) and algae feed/challenged groups. Well-developed melano-macrophage centers are noticed in all groups. H&E X 400

DISCUSSION

Our results showed that the algae feed group had a normal histological status, which may be a sign of *Ulva* algae's safety profile.

Investigating the potential synergistic effect of marine-derived antimicrobial drugs with already-existing ones is another trend driven by the growing risk of multidrug resistance (MDR), which forces the globe to search for and develop natural and effective antimicrobial agents (**Cheung *et al.*, 2014**). Very few researchers have studied Egyptian antibacterial agents of marine origin (**Shanab *et al.*, 2007**; **El Baroty *et al.*, 2011**; **El Baz *et al.*, 2013**; **El Shafay *et al.*, 2016**; **Abdellatief *et al.*, 2019**).

The challenged *O. niloticus* group in this study displayed outward symptoms that were typical of hemorrhagic septicemia (**Abdelsalam *et al.*, 2021; Bakiyev *et al.*, 2022; Farouk *et al.*, 2023)**. There were indications of decline during the postmortem examination, including an enlarged liver and internal organ congestion (**Austin & Austin, 2016; Aboyadak *et al.*, 2017; Hamouda *et al.*, 2019)**, whereas the *Ulva*-treated

group and the *A. hydrophila*-challenged group displayed mild signs of congestion and a low mortality rate.

In the *in vitro* study, *Ulva* algae were recorded with no effectiveness on Gram-positive bacteria, while **Habbu *et al.* (2016**) upon investigating the antimicrobial properties of both the green algae *Ulva lactuca*, its associated endophytic bacteria had effectiveness against *Bacillus subtilis* and *Enterococcus faecalis* (Gram-positive). **El Shoubaky and Salem (2014**) stated that there was no inhibitory impact of the fatty acid extract of *Padina pavonica* (brown algae) on *S. aureus* and *E. coli*. **Ahmed *et al.* (2023**) tested the fatty acid brown macroalgae extracts and recorded the highest antimicrobial activities against *Candida albicans*, *S. aureus*, and *B.subtilis* compared to the crude macroalgal extracts.

Eminently, bacterial toxins are responsible for the increased ALT and AST levels in G2, which lead to oxidative stress causing damage to the liver and muscles, as reported by **El-Barbary (2017**), and in accordance with our histopathological findings. The antioxidant properties of the omega-3 fatty acids found in *Ulva* algae may be the cause of the notable drop in ALT and AST levels in G4 as opposed to G2, as noted by **Sheikhzadehn *et al.* (2024)**.

Systemic inflammation brought on by *A. hydrophila* infection changes lipid metabolism, which may cause the liver to create too many triglycerides as part of the acute-phase reaction (**Zhai *et al.*, 2023**). This could explain the elevated triglyceride levels in G2 compared to G1, which is consistent with the findings of **Dias *et al.* (2023**) in *Arapaima gigas* challenged with *A. hydrophila*.

In general, elevated blood cholesterol is linked to a faster rate of lipid metabolism in bodily tissues, especially during stressful situations, as explained by **Üner (2006)**. This explains the rise in cholesterol levels in G2 compared to G1. Nonetheless, in G4, cholesterol levels returned to almost normal levels, similar to G1. Ulva algae's contribution to the restoration of normal metabolic rates may be the cause of this improvement, as previously mentioned by **Abd-Eldaim *et al.* (2020)**.

Triglyceride levels in G4 were lower than those recorded in G2 after feeding *Ulva* algae, which may be explained by the algae's modulatory effect on lipid metabolism. This finding aligns with the results of **de Assis *et al.* (2020**), who studied the effect of *Aloe vera* on *Piaractus mesopotamicus*, and **Wu *et al.* (2023)**, who investigated the effects of probiotic feeding in *Cyprinus carpio*, *Haematopterus*.

G2 has a lower albumin level than G1, which is explained by liver injury. Such damage impairs the liver's ability to produce albumin, leading to reduced serum levels **(El-Barbary, 2017**). However, compared to G2, *Ulva* algae dramatically raised the albumin

serum levels in G3 and G4. This result is consistent with the findings of **Abdel-Daim *et al*. (2020)** and **Youssef (2023)**, who observed similar effects when feeding *Spirulina platensis*. Since blood albumin is commonly used as an indicator of humoral immunity (**Alexander, 2011**), *Ulva* algae can be considered an immunostimulant.

As part of the immune system's reaction to fight the illness, infections usually cause an increase in particular globulin types, particularly immunoglobulins **(John & Pratt, 2018**). Therefore, infection with *A. hydrophila* is expected to cause an elevation in globulin levels in G2 compared to G1. Furthermore, globulin levels in G4 appeared to be considerably lower than in G2 due to *Ulva alga*e's antimicrobial properties. This finding aligns with the results of **Sattanathan *et al*. (2022)** in *Labeo rohita*.

Biological systems need endogenous antioxidants like CAT and GSH to defend themselves against oxidative stress (**Baldissera *et al*., 2017**). In line with histological findings, our investigation demonstrated that the hepatic and renal CAT and GSH activity was much lower in G2 compared to G1, and that the activity was restored in G4 to a nearly normal level. According to the majority of published research, elevated levels of malondialdehyde (MDA) in the liver and kidneys are indicative of oxidative stress and may indicate infection-related oxidative damage (**Bandeira Junior & Baldisserotto, 2021)**. For instance, *A. hydrophila* infection in the silver catfish has been shown to elevate hepatic MDA levels (**Baldissera *et al*., 2017**). Likewise, in our investigation, MDA levels in G2 were significantly higher than in G1, although MDA levels in G4 were significantly lower and closer to normal. The antioxidant qualities of *Ulva* algae may be responsible for the noted increases in CAT, GSH, and MDA levels in G4, as previously reported by **Akbary and Aminikhoei (2018**).

Our findings are in partial consistency with **Baumgartner *et al*. (2017)**, who described the spleen as one of the primary organs impacted by (motile) *A. hydrophila*, with lesions primarily consisting of necrotic alterations, such as necrosis of ellipsoids and necrotic debris combined with fibrin and amorphous protein. Both the *A.hydrophila*-infected and the algae-feed/challenged groups exhibited these characteristics.

Renal changes in *A.hydrophila* group showed severe degenerative changes of tubular lining epithelia, collapsing of renal glomeruli, and dilation of Bowman’s spaces; this is consistent with **Baumgartner *et al*. (2017)** and **Mazumder *et al*. (2021)**. Our hepatic findings of necrosis and inflammatory cell infiltration were also recorded by **Baumgrtner *et al*. (2017)**. The liver is not the primary target of *A. hydrophila* in the few cases that have been studied.

Petit *et al*. (2023) examined the immune-modulating properties of green and red macroalgal crude extract, attributing this to its higher concentration of marine sulphated polysaccharides. The Nile tilapia and rainbow trout showed comparatively significant

reactive oxygen species (ROS) potential using head kidney leukocytes as a model for immune response estimation, with indications for fish species-specific differences in the impacts of marine sulphated polysaccharide-rich extracts.

Algal fatty acids' antibacterial properties were ascribed to their lytic effect on the bacterial cell wall, which inhibits electron transport and oxidative phosphorylation of the bacterial cell membrane, resulting in ATP transfer. This is followed by an inhibition of bacterial enzymatic activities, primarily for the synthesis of fatty acids in the bacterial cell wall, which causes bacterial cell perforation and cell degradation **(Čermáket *et al.*, 2015)**, which could explain the predominant anti-bacterial effect of G-ve bacteria than G+ve bacteria due to low lipid content and hence more sensitivity for its modulation in G-ve bacteria strains.

Long-chain polyunsaturated fatty acids are responsible for the antibacterial properties of macro-algae fatty acids, such as oleic **(Alamsjah *et al.*, 2007)** and linoleic **(Stabili *et al.*, 2014)** fatty acids. Those acids have been recorded with antibacterial effect through inhibition of bacterial fatty acids synthesis and consequently affecting bacterial external membrane permeability **(Balcázar *et al.*, 2007)**.

The marine algae components of polysaccharides that enhance the hepatic status of some illnesses, such as hepatic steatosis and ballooning, may be responsible for the improvement in hepatic degenerative changes shown in our study **(Zhang *et al.*, 2021)** mediated by increasing hydrogen sulfide production and subsequent reduction of serum glycerol level **(Ren *et al.*, 2018)**. By enhancing the conversion of cholesterol to bile acids and decreasing the reabsorption of bile acids, marine polysaccharides lower the hepatic level of lipids **(Pengzhan *et al.*, 2003; Ma *et al.*, 2022)**. Higher levels of GSH and the antioxidant enzymes GST, GPx, and CAT were linked to the protective action of ulvan polysaccharides, which was demonstrated by decreased lipid peroxides **(Monga *et al.*, 2011)**. The gut microbiota ferments macroalgal polysaccharides, which are difficult to digest, and produces short-chain fatty acids that have the advantage of lowering hepatic lipid synthesis and the liver fatty acid synthase process **(Den Besten *et al.*, 2015)**.

Polysaccharide of *Ulva lactuca* has an immune-regulatory activity as stated **by Zhao *et al.* (2020)**, with upregulation of IgM. In addition, *Ulva* polysaccharides revealed significant stimulatory effects on various immunocytes, such as T cells, B cells, macrophages, and natural killer cells **(Son *et al.*, 2024)** mediated by induction of significant amounts of NO and cytokines, suggesting their strong immune-stimulant effect **(Tabarsa *et al.*, 2012)**. The growth of melano-macrophage centers in the liver and spleen may be explained by these findings.

Melanomacrophage centers (MMCs) are groups of phagocytic cells that can produce melanin in addition to storing hemosiderin and lipofuscin pigment granules.

Melanomacrophages are the follicular dendritic cells, and MMCs are the germinal centers. They are primarily found in the stroma of lymphoid and hematopoietic tissues, including the liver, spleen, and kidney, and they have defense mechanisms mediated by the innate and adaptive immune response **(Stosik *et al*., 2019)**. Other suggestions have been asuumed for melanomacrophages to be resembled to Kupffer cells in mammalian liver **(SmedsrØd, *et al*., 1985)**.

The extent and amount of melano-macrophage centers, which typically contain a variety of pigments, including melanins, increase in older fish and/or when cachectic illness is present. Chronic infections may arise from resistant intracellular bacteria that are focally deposited in melano-macrophage centers. Although iron capture and storage seem to be the main functions in hemolytic diseases, other functions that have been described include antigen presentation and trapping to lymphocytes, sequestration of cellular degradation products, and potentially toxic tissue materials like melanin, free radicals, and catabolic breakdown products. Likewise, it represents a marker for environmental stress **(Agius & Roberts, 2003)**. The MMC, with phagocytic cellular content, contains several types of intracellular pigments: lipofuscin, hemosiderin, and melanin. Because melanin has antioxidant properties and scavenges free radicals, it shields surrounding tissue from oxidative stress **(Scalia *et al*., 1988)** in addition to immune defense function **(Mackintosh, 2001)**. Therefore, melano-macrophage center expansion in *A. hydrophila*-infected, algae-fed, and challenged groups may be a sign of immune modulation, either as a result of infection or as an outcome of algae immune stimulation. **Gao *et al*. (2019)** further supported these findings by examining the immunomodulatory properties of sulfated polysaccharide and polysaccharide iron (III) complex and documenting their stimulation of macrophage activity and lymphocyte proliferation.

Marine algae have often been described as prebiotics with functional dietary elements that support healthy immunological and gastrointestinal function **(Shannon *et al*., 2022)** and inhibit pathogenic microorganisms **(Pung *et al*., 2022)**.

Regarding renal findings, our study detected noticeable improvement in the renal histological status. **Yang *et al*. (2021)** examined *Ulva lactuca* polysaccharide extract's (UPE) potential to mitigate oxidative stress-induced kidney damage. They suggest that UPE may be used as a treatment for kidney damage due to its antioxidant properties. The amount of *Ulva* algae extract in the diet has been recorded with an impact on the upregulation of immune response mediators (TNF-α, NFκ-B, SOD, and COXII) and antioxidants **(Abo-Raya *et al*., 2023)**.

.

CONCLUSION

The study findings stated that whole *Ulva* algae added to the diet at a rate of 1g/ kg feed for four weeks is safe and significantly protects *O. niloticus* from being infected by

A. hydrophila. Improved antioxidant defences, maintained liver and renal function, and decreased mortality are most likely the mechanisms underlying the protective effect. This search reveals that whole marine *Ulva* algae are safe and have antimicrobial protective properties. This is mostly due to their high fatty acid content, which makes them easily usable in fish aquaculture.

REFERENCES

Abdel-Daim, M.M.; Dawood, M.A.O.; Elbadawy, M.; Lotfy, A. and Alkahtani, S. (2020). *Spirulina platensis* reduced oxidative damage induced by chlorpyrifos toxicity in Nile tilapia (*Oreochromis niloticus*). Animals, 10 (3), 473

Abdellatief, J.I.; Salama, S.S.A. and Alkalamawey. N.M. (2019). Preventive effect of *Spirulina platensis* against *Vibrio vulnificus* infection in eels (*Anguilla anguilla*). Animal Health Research Journal Vol. *7*, No. 4; pp.432-444.

Abdelsalam, M.; Ewiss, M.Z.; Khalefa, H.S.; Mahmoud, M.A.; Elgendy, M.Y. and Abdel-Moneam, D.A. (2021). Co-infections of *Aeromonas* spp., *Enterococcus faecalis*, and *Vibrio alginolyticus* isolated from farmed Nile tilapia and African catfish in Egypt, with an emphasis on poor water quality. Microbial Pathogenesis, 160: 105213.

Abdel-Wahab, M.M.; Taha, N.M.; Lebda, M.A.; Elfeky, M.S. and Abdel-Latif, H.M.R. (2021). Effects of bovine lactoferrin and chitosan nanoparticles on serum biochemical indices, antioxidative enzymes, transcriptomic responses, and resistance of Nile tilapia against *Aeromonas hydrophila*. Fish Shellfish Immunol. ;111:160-169. doi: 10.1016/j.fsi.2021.01.017

Abo-Raya, M.H.; Shi, Y.; Wang, Y.; Sayed, S.M. and Shukry, M. (2024). Enhancing immune and antioxidant responses in Nile tilapia through dietary supplementation with Ulva fasciata extract: A study on gene expression and resistance to *Aeromonas hydrophila*. J Anim Physiol Anim Nutr (Berl)., 108(5):1415-1429. doi: 10.1111/jpn.13984.

Aboyadak, I.M.; Ali, N.G.; Goda, A.; Saad, W. and Salam, A.M. (2017). Non-selectivity of RS media for *Aeromonas hydrophila* and TCBS media for Vibrio species isolated from diseased *Oreochromis niloticus*. J. Aquac. Res. Dev., 8(496): 1-5.

Aebi, H. 1984. Catalase in Vitro. Methods Enzymology, 105, 121-126.

Agius, C. and Roberts, R.J. (2003): Melano-macrophage centres and their role in fish pathology. J Fish Dis., 26(9):499-509. doi: 10.1046/j.1365-2761.2003.00485.x.

Ahmed, H.; Heneidak, S.; El Shoubaky, G.A. and Rasmey, M.A.(2023).In Vitro Comparative Antimicrobial Potential of Bioactive Crude and Fatty acids Extracted from Abundant Marine Macroalgae, Egypt. Frontiers in Scientific Research and Technology, 7 : 11 - 20 10.21608/FSRT.2023.221539.1097

Akbary, P. and Aminikhoei, Z. (2018) "Effect of water-soluble polysaccharide extract from the green alga *Ulva rigida* on growth performance, antioxidant enzyme

activity, and immune stimulation of grey mullet *Mugil cephalus*." Journal of applied phycology, 30 : 1345-1353.

Alamsjah, M.A.; Ishibe, K.; Kim, D.; Yamaguchi, K.; Ishibashi, F.; Fujita, Y. and Oda, T. (2007): Selective toxic effects of polyunsaturated fatty acids derived from *Ulva fasciata* on red tide phytoplankter species. Biosci Biotechnol Biochem., 71(1):265-8. doi: 10.1271/bbb.60475.

Alexander, C.; Sahu, N.P.; Pal, A.K. and Akhtar, M.S. (2011). Haemato-immunological and stress responses of *Labeo rohita* (Hamilton) fingerlings: Effect of rearing temperature and dietary gelatinized carbohydrate. J. Anim. Physiol. Anim. Nutr., 95:653–663. doi: 10.1111/j.1439-0396.2010.01096.x. [PubMed] [CrossRef] [Google Scholar

Anantasuk, N.; Phurahong, T.; Pumchan, A.; Hirono, I. and Unajak, S. (2024). Molecular characterization and bivalent vaccine development of *Aeromonas hydrophila* and *Aeromonas ve-ronii* in Nile tilapia (*Oreochromis niloticus*). Aquaculture, 590: 741042.

Austin, B. and Austin, D.A. (2016). Aeromonadaceae representatives (motile aeromonads). Bacterial fish pathogens: Disease of farmed and wild fish, pp.119-146. https://link.springer.com/chapter/10.1007/978-3-319-32674-0_4

Ayoub, H.F.; Khafagy, A.R.; Esawy, A.M.; El-Moaty, N.A., Alwutayd, K.M., Mansour, A.T. and El-Tarabili, R.M. (2024). Phenotypic, molecular detection, and antibiotic resistance profile (MDR and XDR) of *Aeromonas hydrophila* isolated from farmed *Tilapia zillii* and *Mugil cephalus*. BMC Veterinary Research, 20(1):

Bakiyev, S.; Smekenov, I.; Zharkova, I.; Kobegenova, S.; Sergaliyev, N.; Absatirov, G. and Bissenbaev, A. (2022). Isolation, identification, and characterization of pathogenic *Aeromonas hydrophila* from critically endangered *Acipenser baerii*. Aquaculture Reports, 26: 101293.1-

Balcázar, J. L.; Rojas-Luna, T. and Cunningham, D. P. (2007): Effect of the addition of four potential probiotic strains on the survival of pacific white shrimp (Litopenaeus vannamei) following immersion challenge with *Vibrio parahaemolyticus*. *Journal of Invertebrate* Pathology, 96(2):147–150. doi: 10.1016/j.jip.2007.04.008.

Baldissera, M.D.; Souza, C.F.; Bandeira Junior, G.; de Vargas, A.P.C.; Boligon, A.A.; de Campos, M.M.A.; Stefani, L.M. and Baldisserotto, B. (2017) *Melaleuca alternifolia* essential oil enhances the non-specific immune system and prevents oxidative damage in *Rhamdia quelen* experimentally infected by *Aeromonas hydrophila*: effects on cholinergic and purinergic systems in liver tissue. Fish Shellfish Immunol., 61, 1–8.]

Bandeira, Junior G, Baldisserotto, B. (2021). Fish infections associated with the genus Aeromonas: a review of the effects on oxidative status. J Appl Microbiol., 131(3):1083-1101. doi: 10.1111/jam.14986. Epub 11. PMID: 33382188.

Baumgartner, W.A.; Ford L. and Hanson, L. (2017): Lesions caused by virulent *Aeromonas hydrophila* in farmed catfish (*Ictalurus punctatus* and *I. punctatus* × I. furcatus) in Mississippi. Journal of Veterinary Diagnostic Investigation, 29(5):747-751. doi: 10.1177/1040638717708584.

Beutler, E.; Duron, O. and Kelly, B.M.(1963). Improved method for the determination of blood glutathione.. The Journal of Laboratory and Clinical Medicine, 61.

Brooks, G.; Carroll, K.; Butel, J.; Morse, S. and Mietzner, T. (2015). Jawetz, Melnick, Adelberg Medical Microbiology. McGraw Hill Medical, New York.

Bu, X.; Lian, X.; Wang, Y.; Luo, C.; Tao, S.; Liao, Y.; Yang, J.; Chen, A. and Yang, Y.(2019). Dietary yeast culture modulates immune response related to TLR2-MyD88-NF-kβ signaling pathway, antioxidant capability and disease resistance against *Aeromonas hydrophila* for Ussuri catfish (*Pseudobagrus ussuriensis*). Fish Shellfish Immunol., 84:711-718. Do13-i: 10.1016/j.fsi..10.049.

Čermák, L.; Pražáková, Š.; Marounek, M.; Skřivan, M., Skřivanová, E. (2015): Effect of green alga *Planktochlorella nurekis* on selected bacteria revealed antibacterial activity *in vitro*. Czech J. Anim. Sci., 60:427–435. doi: 10.17221/8522-CJAS.

Cheung, R.C.F.; Wong, J.H.; Pan, W.L.; Chan, Y.S.; Yin, C.M.; Dan, X.L.; Wang, H.X.; Fang, E.F.; Lam, S.K.; Ngai, P.H.K.l .; Xia LX, Liu F, Ye XY, Zhang GQ, Liu QH, Sha O, Lin P, Ki C, Bekhit AA, Bekhit Ael-D, Wan DC, Ye XJ, Xia J, Ng TB.(2014): Antifungal and antiviral products of marine organisms. Appl. Microbiol. Biotechnol., 98:3475–3494. doi: 10.1007/s00253-014-5575-0. PMID: 24562325; PMCID: PMC5300219.

de Assis, R.W.S. and Urbinati, E.C.(2020). Physiological activity of *Aloe vera* in pacu (*Piaractus mesopotamicus*) inoculated with *Aeromonas hydrophila*. Fish Physiol Biochem 46, 1421–1430 . https://doi.org/10.1007/s10695-020-00800-0

Den Besten, G.; Bleeker, A.; Gerding, A.; van Eunen, K.; Havinga, R.; van Dijk, T.H.; Oosterveer, M.H.; Jonker, J.W.; Groen, A.K.; Reijngoud, D.J., et al. (2015): Short-Chain Fatty Acids Protect Against High-Fat Diet-Induced Obesity via a PPARγ-Dependent Switch from Lipogenesis to Fat Oxidation. Diabetes, 64:2398–2408. doi: 10.2337/db14-1213.

Dias, M. K. R.; Yoshioka, E. T. O.; Rodriguez, A. F. R.; Ribeiro, R. A.; Fernandes, C. P.; Ozório, R. O. A. and Tavares-Dias, M. (2023). *Mansoa alliacea* extract improves the growth performance and innate immune response of *Arapaima gigas* challenged with *Aeromonas hydrophila* and handling stress. Acta Amazonica, 53(1), 24-31

El Baroty, G.S., El-Baz, F.K., Abd-Elmoein, I.;, Abd El Baky Hanaa, H., Ali, M.M. and Ibrahim, E.A. (2011): Evaluation of glycolipids of some Egyptian marine algae as a source of bioactive substances. Int. Res. J. Pharm., 2, 165–174.

El Baz, F.; El Baroty, G.; Abd El Baky, H.; Abd El-Salam, O. and Ibrahim, E. (2013): Structural characterization and biological activity of sulfolipids from selected marine algae. Grasas Aceites, 64, 561–571.

El Shafay, S.M.; Ali, S.S. and El-Sheekh, M.M. (2016): Antimicrobial activity of some seaweed species from Red sea, against multidrug-resistant bacteria. Egypt. J. Aquat. Res., 42, 65–74.

El Shoubaky, G. A.and Salem, E. A. (2014). Active ingredients fatty acids as antibacterial agent from the brown algae *Padina pavonica* and *Hormophysa triquetra*. J. Coastal Life Med., 2(6): 431-438.

El-Barbary, M. I.. (2017). "Serum biochemical and histopathological changes associated with *Aeromonas hydrophila* isolated from *Oreochromis niloticus* and *Sparus aurata* with multiple antibiotic resistance index. Journal of Biological Sciences", 17(5): 222-234

Eurell, T.E.; Lewis, S.D.H. and Grumbles, E.C. (1978). Comparison of selective diagnosis tests for detection of motile *Aeromonas septicaemia* in fish. Am. J. Vet. Res., 39(8): 1384-1386.

FAO (2002). Antibiotic residues in aquaculture products. The State of World Fisheries and Aquaculture. Food and Agriculture Organisation of the United Nations, 74-83.

Farouk, G.M.; Salama, S.S.A and Abdelaziz, M.A.M. (2023). Prevalence of aerolysin and hemolysin virulence genes and antibacterial resistance profile to control motile *Aeromonas* septicemia (MAS) isolated from *Oreochromis niloticus* in the Nile River, Egypt. Bulletin of Faculty of Science, Zagazig University (BFSZU), (2): 132-141.

Gao, X.; Qu, H.; Gao, Z.; Zeng, D.; Wang, J.; Baranenko, D.; Li, Y. ad Lu, W. (2019): Protective effects of Ulva pertusa polysaccharide and polysaccharide iron (III) complex on cyclophosphamide-induced immunosuppression in mice. Int J Biol Macromol., 15;133:911-919. doi: 10.1016/j.ijbiomac.2019.04.101.

Gerstel, J.; Turner, T.; Ruiz, G.; Wise, J.; Stein, A.; Jones, G.; Morin, T.; Pinazza, T.; Sukhorukov, E.; Clark, D.; Steen, T.; Wright, B. and Langland, J. (2018): Identification of botanicals with potential therapeutic use against methicillin-resistant *Staphylococcus aureus* (MRSA) infections. Phytother. Res., 32(12):2577–2585. doi: 10.1002/ptr.6198.

Habbu, P.;Warad, V.; Shastri1, R.; Savant, C.; Madagundi, S. and Kekare, P. (2016): In vitro and in vivo antimicrobial activity of *Ulva lactuca* Linn. (Green algae) associated endophytic bacterial Strains. J. Appl. Pharm. Sci., 6(10):138–146.

Hamouda, A.; Moustafa, E. and Zayed, M. (2019). Overview on the most prevailing bacterial diseases infecting *Oreochromis niloticus* at Aswan fish hatchery, Egypt. Adv. Anim. Vet. Sci., 7(11): 950-961.

Hemaiswarya, S.; Kruthiventi, A.K. and Doble, M. (2008).Synergism between natural products and antibiotics against infectious diseases. Phytomedicine. 15:639–652

Hou, T.; Liu, H. and Li, C.(2022) Traditional Chinese herb formulas in diet enhance the non-specific immune responses of yellow catfish (*Pelteobagrus fulvidraco*) and resistance against *Aeromonas hydrophila*. Fish Shellfish Immunol., 131:631-636. doi: 10.1016/j.fsi.10.050.

Ismail, A., Ktari, L.; Ben Redjem R. Y.; Brahim,A.; Saloua, S.; Abdellatif, B. and El Bour M. (2018). Antimicrobial Fatty Acids from Green Alga Ulva rigida (Chlorophyta). *BioMed Research International* Volume 2018, Article ID 3069595, 12 pages .**https://doi.org/10.1155/2018/3069595**

Janda, J. M. and Abbott, S. L. (2010). The genus Aeromonas: taxonomy, pathogenicity, and infection. Clinical Microbiology Reviews, 23(1): 35-73.

John, S. and Pratt, D. S. "Harrison's principles of internal medicine 20th edition." (2018): 276.

Karunasagar, I.; Pai, R.; Malahti, G.R. and Karunasagar, I.(1994) Mass mortality of *Penaeus monodon* larvae due to antibiotic-resistant *Vibrio harveyi* infection. *Aquaculture, 128*:203-209.

Khameneh, B.; Iranshahy, M.; Soheili, V. and Fazly, B. B.S. (2019). Review on plant antimicrobials: a mechanistic viewpoint. Antimicrob Resist Infect Control, **8**: 118 https://doi.org/10.1186/s13756-019-0559-6

Ma, Y.; Liu, X.; Liu, D.; Yin, Z.; Yang, X. and Zeng, M. (2022): Oyster (*Crassostrea gigas*) Polysaccharide Ameliorates High-Fat-Diet-Induced Oxidative Stress and Inflammation in the Liver via the Bile Acid-FXR-AMPKα Pathway. J. Agric. Food Chem., *70*:8662–8671. doi: 10.1021/acs.jafc.2c02490.

Mackintosh, J.A. (2001).The antimicrobial properties of melanocytes; melanosomes and melanin and the evolution of black skin. J. Their Biol., 211;101-113.

Manivasagan, P.; Kang, K.H.; Sivakumar, K.; Li-Chan, E.C.; Oh, H.M. and Kim, S.K.(2014). Marine actinobacteria: An important source of bioactive natural products. *Environ.* Toxicol. Pharmacol.; 38:172–188.

Mazumder, A.; Choudhury, H.; Dey, A. and Sarma, D. (2021): Isolation and characterization of two virulent Aeromonads associated with haemorrhagic septicaemia and tail-rot disease in farmed climbing perch Anabas testudineus. Sci Rep., 11, 5826. https://doi.org/10.1038/s41598-021-84997-x

Monga, J.; Chauhan, C.S. and Sharma, M. (2011): Human epithelial carcinoma cytotoxicity and inhibition of DMBA/TPA induced squamous cell carcinoma in Balb/c mice by Acacia catechu heartwood. J Pharm Pharmacol., 63(11):1470–1482. doi: 10.1111/j.2042-7158.2011.01354.x.

Paray, B.A.; Adeshina, I.; Bhat, E.A.; Raji, A.; Umma; S.B.; Alliy, R.O. and Tiamiyu, L.O. .(2024) Effect of dietary *Lagnaria breviflora* leaves extract on the growth performance, haematological, antioxidative and immune responses of African catfish (*Clarias gariepinus*) juveniles. J Anim Physiol Anim Nutr., 108:1164–1175

Pasdaran, A. and Hamedi, **A**.(2017). Chapter 14—Natural products as source of new antimicrobial compounds for skin infections. In: Kon K., Rai M., editors. *The Microbiology of Skin, Soft Tissue, Bone and Joint Infections.* Academic Press; Cambridge, MA, USA:. pp. 223–253.

Peace, A.J. and Kaplan ,LA.(1987).Clinical chemistry.Mosby st.louis.USA.

Pengzhan, Y.; Ning, L.; Xiguang, L.; Gefei, Z.; Quanbin, Z. and Pengcheng, L. (2003): Antihyperlipidemic effects of different molecular weight sulfated polysaccharides from *Ulva pertusa* (Chlorophyta). Pharmacol. Res., 48:543–549. doi: 10.1016/S1043-6618(03)00215-9.

Petit, J.; den Brink, E.; Collén, P.N.; Haenen, O.L.M.; Schrama, J. and Wiegertjes, G.F. (2023): Green and red macro-algae extracts show antibacterial effects and induce innate immune responses in Nile tilapia and rainbow trout *in vitro*. Comp Immunol Rep., 22(6):200128. doi: 10.1016/j.cirep..200128.

Phomkaivon, N.; Pongponpai, P.; Kosawatpat, P.; Thongdang, B.; Pan-utai, W. Extraction, Characterisation and Evaluation of Antioxidant and Probiotic Growth Potential of Water-Soluble Polysaccharides from *Ulva rigida* Macroalgae. Foods, 13, 1630. https://doi.org/10.3390/foods13111630

Public Health Agency of Canada (2019). Public Health Agency of Canada, published: -12-13, ISSN: 2562-2374.

Pung, H.C.; Lin, W.S.; Lo, Y.C.; Hsu, C.C.; Ho, C.T. and Pan, M.H.(2022): Ulva prolifera polysaccharide exerts anti-obesity effects via upregulation of adiponectin expression and gut microbiota modulation in high-fat diet-fed C57BL/6 mice. J. Food Drug Anal., 30:46–61. doi: 10.38212/2224-6614.3395.

Reitman, S., Frankel, S. (1975.) A colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases. Am. J. Clin. Pathol., 28(1): 56-63

Ren, R.; Yang, Z.; Zhao, A.; Huang, Y.; Lin, S.; Gong, J.; Chen, J.; Zhu, P.; Huang, F. and Lin, W. (2018): Sulfated polysaccharide from *Enteromorpha prolifera* increases hydrogen sulfide production and attenuates non-alcoholic fatty liver disease in high-fat diet rats. Food Funct., 9:4376–4383. doi: 10.1039/C8FO00518D.

Satoh, K. (1978). Serum lipid peroxide in cerebrovascular disorders determined by a new colorimetric method. Clin Chim Acta. 90(1):37-43.

Sattanathan, G.; Liu, W. C.; Padmapriya, S.; Pushparaj, K.; Sureshkumar, S.; Lee, J. W.; Balasubramanian, B and Kim, I. H. (2022). Effects of dietary blend of

algae extract supplementation on growth, biochemical, haemato-immunological response, and immune gene expression in *Labeo rohita* with *Aeromonas hydrophila* post-challenges. Fishes, 8(1), 7

Scalia, M.; Geremia, E.; Corsaro, C.; Santoro, C.; Sciuto, S. and Sichel, G. (1988): The extracutaneous pigmentary system: Evidence for the melanosynthesis in Amphibia and Reptilia. Comp Biochem Physiol., 89B:715-717.

Schaperclaus. (1992). Fish diseases, 1. CRC.

Shanab, S.M. (2007): Antioxidant and antibiotic activities of some seaweeds (Egyptian isolates). Int. J. Agric. Biol., *9*, 220–225.

Shannon, E and Abu-Ghannam, N. (2016): Antibacterial Derivatives of Marine Algae: An Overview of Pharmacological Mechanisms and Applications. Mar Drugs, 14(4): 81.

Shannon, E.; Conlon, M. and Hayes, M. (2022). The Prebiotic Effect of Australian Seaweeds on Commensal Bacteria and Short Chain Fatty Acid Production in a Simulated Gut Model. Nutrients, 14(10):2163. 23. doi:10.3390/nu14102163

Sheikhzadeh, N.; Soltani, M.; Heidarieh, M., and Ghorbani, M. (2024). Role of Dietary Microalgae on Fish Health and Fillet Quality: Recent Insights and Future Prospects. *Fishes*, *9*(1), 26. https://doi.org/10.3390/fishes9010026

Sherif, A.H. and Kassab, A.S. (2023). Multidrug-resistant Aeromonas bacteria prevalence in Nile tilapia broodstock. BMC Microbial., 23(1): 80. https://doi.org/10.1186/s12866-023-02827-8

Siddik, M.A.B.; Francis, P.; Rohani, M.F.; Azam, M.S.; Mock, T.S. and Francis, D.S. (2023): Seaweed and Seaweed-Based Functional Metabolites as Potential Modulators of Growth, Immune and Antioxidant Responses, and Gut Microbiota in Fish. Antioxidants (Basel), 1;12(12):2066. doi: 10.3390/antiox12122066

Sittien, A.; Abdellatief, J.I.; Salama, S.S.; Elkenany, R. and Awad, A. (2024). *Aeromonas hydrophila* Isolated from the River Nile *Oreochromis niloticus*: Molecular Characterization and Antimicrobial Susceptibility. Egyptian Journal of Aquatic Biology & Fisheries, 28(6): 1843 – 1858. ISSN 1110 – 6131.

SmedsrØd, B.; Johansson, S. and Pertoft, H. (1985): Studies in vivo and in vitro on the uptake and degradation of soluble collagen alpha 1(I) chains in rat liver endothelial and Kupfer cells. Biochem J., 228, 415–424.

Son, S.U.; Kim, T.E.; Park, J.H.; Suh, H.J. and Shin, K.S. (2024): Immunostimulating effects of ulvan type polysaccharide isolated from Korean *Ulva pertusa* in cyclophosphamide-induced immunosuppressed BALB/c mice. Int. J. Biol Macromol., *275*(Pt 1):133518. doi: 10.1016/j.ijbiomac.

Srinivasan, R.; Kannappan, A. Shi, Ch. and Lin, X. (2021). Marine Bacterial Secondary Metabolites: A Treasure House for Structurally Unique and Effective Antimicrobial Compounds. Mar Drugs, 19(10): 530.

Stabili, L.; Acquaviva, M. I.; Biandolino, F.; Cavallo, R.A.; De Pascali , S.A.; Fanizzi, F.P.; Narracci, M.; Cecere, E.; Petrocelli , A. (2014): Biotechnological potential of the seaweed *Cladophora rupestris (Chlorophyta, Cladophorales*) lipidic extract. New Biotechnology, 31(5):436–444. doi: 10.1016/j.nbt.

Stefanović, O.D.(2018). Synergistic activity of antibiotics and bioactive plant extracts: A study against Gram-positive and Gram-negative bacteria. In: Kırmusaoğlu S., editor. *Bacterial* Pathogenesis and Antibacterial Control. Intech Open; London, UK: pp. 23–48.

Stosik, M.P.; Tokarz-Deptuła, B. and Deptuła, W. (2019): Melanomacrophages and melanomacrophage centres in Osteichthyes. Cent Eur J Immunol., 44(2):201-205. doi: 10.5114/ceji.2019.87072.

Suvarna, K.S.; Layton, C. and Bancroft, J.D. (2012). Bancroft's Theory and Practice of Histological Techniques: Expert Consult: Online and Print. Elsevier Health Sciences.

Tabarsa, M.; Han, J.H.; Kim, C.Y. and You, S.G. (2012): Molecular characteristics and immunomodulatory activities of water-soluble sulfated polysaccharides from Ulva pertusa. J Med Food, 15(2):135-44. doi: 10.1089/jmf.2011.1716.

Tietz, N.W. (1986) Textbook of clinical chemistry. 1919.Philadelphia: WB Saunders.

Tietz, N.W. (1994) Textbook of clinical chemistry. 2nd ed. Philadelphia: WB Saunders.

Üner, N.; Oruç, E.Ö.; Sevgiler, Y.; Şahin, N.; Durmaz, H. and Usta, D.(2006). Effects of diazinon on acetylcholinesterase activity and lipid peroxidation in the brain of *Oreochromis niloticus*. Environ. *Toxicol. Pharmacol*., 21:241–245. doi: 10.1016/j.etap.. [PubMed] [CrossRef] [Google Scholar]

Wahlefeld, A.W. (1974). Cloremetric determination of enzymatic triglycol in serum. In: M.M. Bermeyer (eds), Methodes of Enzymatic Analysis. Acad. Press, N. Y., 5: 1831-1835. https://doi.org/10.1016/B978-0-12-091304-6.50036-7

Watson, D. (1960). A simple method for the determination of serum cholesterol. Clin. Chim. Acta., 5: 637-643. https://doi.org/10.1016/0009-8981(60)90004-8

World Health Organization No Time to Wait (2020) Securing the Future from Drug-Resistant Infections. Report to the Secretary-General of the United Nations.

Wu, L.; Wang, L.; Cui, S.; Peng, Z.; Liu, Z.; Li, M., ... and Ren, T. (2023). Effects of dietary compound probiotics and heat-killed compound probiotics on antioxidative capacity, plasma biochemical parameters, intestinal morphology, and microbiota of *Cyprinus carpio* Haematopterus. Aquaculture International, 31(4), 2199-2219.

Wybenga, D.R.; Di Giorgio, J. and Pileggi, V.J. (1971). Manual and automated methods for urea nitrogen measurement in whole serum. Clin. Chem., 17(9): 891-895.

Yang, Q.; Jiang, Y.; Fu, S.; Shen, Z.; Zong, W.; Xia, Z.; Zhan, Z and Jiang, X. (2021): Protective Effects of Ulva lactuca Polysaccharide Extract on Oxidative Stress and Kidney Injury Induced by D-Galactose in Mice. Marine drugs, 19(10), 539. https://doi.org/10.3390/md19100539

Yoon, B.K.; Jackman, J.A.; Valle-González, E.R. and Cho, N.J. (2018). Antibacterial free fatty acids and monoglycerides: Biological activities, experimental testing, and therapeutic applications. Int. J. Mol. Sci., 19:1114.

Young, D.S. (1997). Effects of drugs on clinical laboratory tests. Ann. Clin. Biochem., 34 (6): 579.

Youssef, I.M.I.; Saleh, E.S.E.; Tawfeek, S.S.; Abdel-Fadeel, A.A.A.; Abdel-Razik, A.H. and Abdel-Daim, A.S.A.920230. Effect of *Spirulina platensis* on growth, hematological, biochemical, and immunological parameters of Nile tilapia (*Oreochromis niloticus*). Trop Anim Health Prod 27,55(4):275. doi: 10.1007/s11250-023-03690-5. PMID: 37498411; PMCID: PMC10374666.

Zhai, W.; Wang, Z.; Ye, C.; et al. (2023). IL-6 Mutation Attenuates Liver Injury Caused by *Aeromonas hydrophila* Infection by Reducing Oxidative Stress in Zebrafish. International Journal of Molecular Sciences, 24(24):17215. DOI: 10.3390/ijms242417215. PMID: 38139043; PMCID: PMC10743878

Zhang, Y.; Yang, L.; Zhao, N.; Hong, Z.; Cai, B.; Le, Q.; Yang, T.; Shi, L. and He, J. (2021). Soluble Polysaccharide Derived from *Laminaria japonica* Attenuates Obesity-Related Nonalcoholic Fatty Liver Disease Associated with Gut Microbiota Regulation. Mar. Drugs,19:699. doi: 10.3390/md19120699.

Zhao, C.; Lin, G.; Wu, D.; Liu, D.; You, L.; Högger, P.; ... and Xiao, J. (2020): The algal polysaccharide ulvan suppresses growth of hepatoma cells. Food Frontiers, 1(1), 83-101.