Aquaculture International
https://doi.org/10.1007/s10499-024-01564-7

RESEARCH

Efficacy of the dietary *Malva parviflora* leaves in modulating immune-antioxidant functions, resistance to *Candida albicans* and *Streptococcus agalactiae* infection, digestive/absorptive capacity, and growth of Nile tilapia (*Oreochromis niloticus*)

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Received: 6 March 2024 / Accepted: 29 May 2024

Abstract
Recently, the intervention of herbal plants in the fish diet has drawn a lot of interest due to their unique nutritional value and pharmacological properties. To assess the impact of dietary incorporation of Egyptian mallow, *Malva parviflora* leaves powder (MPLP), a 10-week feeding trial was carried out on Nile tilapia (*Oreochromis niloticus*). The assessment encompassed the effects on growth, digestive/absorptive efficiency, biochemical, and immuno-antioxidant responses. Four treatments containing a total of 200 fish ($n=50$ fish/treatment; five replicates/treatment; 10 fish/replicate) with an average weight of 37.50 ± 0.22 g (means $\pm$ *SE*) were randomly assigned. The MPLP was added to the basal diet at 0 (control diet, MPLP0), 5 (MPLP5), 10 (MPLP10), and 15 (MPLP15) g/kg diet concentrations and served to the fish for 10 weeks. After the trial, the fish were exposed to pathogenic yeast (*Candida albicans*) and bacterium (*Streptococcus agalactiae*) infection. Results displayed that MPLP-incorporated diets significantly ($P<0.05$) enhanced the growth, intestinal digestive enzymes (amylase and lipase), and intestinal morphometrics (villous length and width and goblet cell count). The increases were in a level-dependent way, and the MPLP15 diet showed the highest values. Dietary MPLP notably ($P<0.05$) enhanced growth hormone and declined leptin hormone, glucose, and cholesterol levels. Moreover, the immunological (serum bactericidal activity, myeloperoxidase, and complement 3) and antioxidant (catalase, glutathione peroxidase, and total antioxidant capacity) biomarkers were augmented in the MPLP-fed groups in a level-dependent way ($P<0.05$). Fifteen days post-infection with *C. albicans* and *S. agalactiae*, the survival rates among the infected fish enhanced as dietary MPLP levels rose; the MPLP15 group had the highest survival rates (90.91 and 81.82%), respectively. Overall, dietary MPLP intervention can boost the growth, digestive capacity, disease resistance, and immune-antioxidant status of Nile tilapia. These positive effects may contribute to a sustainable aquaculture industry.

Handling Editor: Daniel Merrifield

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Keywords Disease resistance · Feed additives · Immune status · Intestinal morphology · *Malva parviflora* · *Oreochromis niloticus*

Introduction

Aquaculture has become one of the most attractive agricultural sectors throughout the years. This is because of the rising demand for fish due to the increasing global population (FAO 2023; Bjørndal et al. 2024). The Nile tilapia (*Oreochromis niloticus*) belongs to the Cichlidae family and is the second most commonly grown fish species globally because of its high-quality fish protein (El-Sayed 2019). The harvest of Nile tilapia reached over 6 million tonnes worldwide, and further expansion is predicted (FAO 2020; Boyd et al. 2022). To achieve large yields, farmers typically choose to intensify fish production. However, this intensification is usually associated with disease outbreaks due to unsustainable practices and harsh conditions that lead to economic losses (Ibrahim et al. 2022; Mahboub et al. 2022).

Fungal and bacterial infections are among the main causes of the massive losses that face the fish farming sector annually (Abd El-Hack et al. 2022). Fungi, particularly more succinctly, *Candida albicans*, are known to assault fish and cause significant losses in aquaculture (Iqbal and Saleemi 2013). The infection with *C. albicans* can result in numerous skin sores, fish mortality, and subsequent bacterial invasion in the fish (Eissa et al. 2013; Mahboub et al. 2024). A pathogenic Gram-positive bacterium called *Streptococcus* spp. is among the invading bacterial infections in aquaculture. They have the potential to infect a wide variety of fish species globally and cost the aquaculture industry millions of dollars in lost revenue (Salazar et al. 2016; Van Doan et al. 2022). *Streptococcus agalactiae* is a widely distributed species worldwide (Ghetas et al. 2021). *S. agalactiae* induces ascites, meningoencephalitis, septicemia, exophthalmia, and even mortalities in fish (Rivas et al. 2023). Its infection has previously posed a major threat to Nile tilapia's health and productivity (Baldissera et al. 2020; Abdel-Tawwab et al. 2023).

As a result, improving fish health is crucial to preventing disease outbreaks. Nutritional manipulation is a highly effective and pragmatic approach for promoting fish growth and well-being (Dara et al. 2023). A variety of studies on the use of different immuno-stimulants including herbal plants in fish feeds as growth promoters and health boosters have been conducted over the past 20 years (Reverter et al. 2021; Aly et al. 2023). Moreover, these plants or their byproducts can serve as affordable, safe, and efficient substitutes for antimicrobials (Rahimi et al. 2022). There is still much to learn about the effects of medicinal plants on fish despite these numerous studies.

A member of the family Malvaceae is *Malva parviflora* which is a perennial or annual plant. It can be found extensively throughout Europe and in North Africa (Maity et al. 2018). It is locally known as khabazi in Egypt, and it also has several common names, including Egyptian mallow, common mallow, and cheeseweed (Munir et al. 2021). The most often utilized plant parts are the leaves and flowers. *M. parviflora* leaves with a pleasant taste have pharmacological potentials such as antimicrobial, antioxidant, neuroprotective, analgesic, antipyretic, anti-irritant, and anti-inflammatory (Azab 2017; Singh and Navneet 2017). These benefits are because of the highest concentration of flavonoids, phenols, carotenoids, unsaturated fatty acids, and minerals (Farhan et al. 2012; Abd El-Salam and Morsy 2019).

Few studies have been done on using *M. parviflora* as a dietary supplement on Nile tilapia diets. However, there is still a lack of information about the dietary use of its leaves in fish regarding enhancing digestive/absorptive capability and intestinal morphometrics and other immunological and stress-related biomarkers. Therefore, the present perspective investigated the promising potentials of dietary *M. parviflora* leaves powder (MPLP) on digestion, intestinal histomorphology (indicative of the effectiveness of food absorption and the gut's immunity), and the growth rate of *O. niloticus*. As well, the resistance against the pathogenic yeast (*C. albicans*) and bacterium (*S. agalactiae*) infection was investigated.

Materials and methods

Preparation of MPLP and tested diets

The Desert Research Center, Egypt, provided the *M. parviflora* leaves. After washing and drying in the shade at 25 °C, the leaves were ground into fine particles. MPLP was mixed with the components of the basal diet at 0, 5, 10, and 15 g/kg diet concentrations (MPLP0, MPLP5, MPLP10, and MPLP15), respectively. The dietary ingredients were prepared to fulfill *O. niloticus* nutritional requirements (El-Sayed and Teshima 1992; NRC 2011), mechanically mixed, pelletized (1.5 mm), and air-dried at 25 °C for 24 h before being kept in storage at 4 °C until required. As per the guidelines provided by the AOAC (2005), the proximate analysis of the basal diet was carried out (Table 1).

Table 1 Proximate chemical composition of basal diet (% on dry basis)

	%
Ingredients	
Corn gluten 60% CP	11
Ground yellow corn	24.3
Fish meal	18
Fish oil	6
Soybean meal 44%	25.5
Wheat bran	9
Wheat	5
Premix[#]	1.2
Calculated chemical analysis	
Crude fat	9.46
Crude protein	33.62
Crude fiber	3.74
NFE[##]	38.56
Digestible energy (Kcal/kg)	2907.3

[#]Each 1 kg of premix has vitamin D, 110,000 IU; vitamin A, 550,000 IU; vitamin K, 484 mg; vitamin C, 50 g; vitamin E, 11,000 mg; choline, 110,000 mg; vitamin B_2, 660 mg; vitamin B_1, 440 mg; vitamin B_5, 1100 mg; vitamin B_9, 55 mg; vitamin B_3, 13,200 mg; vitamin B_6, 1045 mg; selenium, 44 mg; manganese, 1320 mg; copper, 330 mg; biotin, 6.6 mg; iodine, 110 mg; iron, 6.6 g; and zinc, 6.6 g

[##]NFE: nitrogen-free extract = 1000 − (g/kg crude fat + crude protein + crude fiber + ash)

Research ethics and fish rearing

This work was accepted by the Zagazig University Authority for Animal Use in Research (ZU-IACUC-2-F-2-2024). Fish were supplied from a private fish farm in Kafr El-Sheik Governorate, Egypt. The fish were thoroughly inspected to determine their health in compliance with CCAC (2005) guidelines. The fish were randomly placed in 100-L dechlorinated water static glass aquariums (ten fish per aquarium). The aquarium was supplied with air pumps and a 12-h cycle fluorescent light source. Before the experiment commenced, fish were allowed a 2-week period to acclimatize to the lab setting. Fish were hand-fed the basal diet until they reached visual satiation during the acclimation time. After weighing each fish individually, the average initial weight (IBW) was 37.50 ± 0.22 g (means ± *SE*).

Eliminating the fish waste from the bottom of the tank by siphoning, changing out three-quarters of the aquarium's water, and adding fresh water were all part of the daily hygienic procedures. The whole water was changed twice a week. Throughout the trial, the temperature, ammonia, dissolved oxygen, nitrite, and pH were 23.3 ± 1.5 °C, 0.01 ± 0.002 mg/L, 6.72 ± 0.27 mg/L, 0.03 ± 0.01 mg/L, and 6.40 ± 0.21, respectively. These variables were estimated using the most recent criteria APHA (2005) and were within the limits permitted for the culturing of species (Boyd and Tucker 2012). MPLP addition did not significantly change the results.

Experimental strategy and evaluation of survival rate and growth metrics

For 10 weeks, the fish ($n = 50$ fish/group) were randomly assigned into four groups (five replicates/group; ten fish/replicate). Fish were fed on the tested diets (MPLP0, MPLP5, MPLP10, and MPLP15), where MPLP0 served as the control diet. Three times a day (9:00, 12:00, and 15:00), fish were hand-fed the diets until they reached visual satiation.

At the end of the trial (10 weeks), the total feed intake (TFI) and final body weight (FBW) were assessed. The survival rate and growth/feed utilization metrics were estimated (Abdel Rahman et al. 2021). They included body weight gain (BWG), specific growth rate (SGR), protein efficiency ratio (PER), and feed conversion ratio (FCR) following these equations:

$$\text{Survival rate } (\%) = 100 \times \frac{\text{Final number of fish at the end of the feeding trial}}{\text{The initial number of fish}}$$

$$\text{TFI/ fish (g)} = \frac{\text{Totalconsumed feed (g)/tank}}{\text{Number of fish}}$$

$$\text{BWG/fish(g)} = \frac{\text{FBW(g)/tank} - \text{IBW(g)/the same tank}}{\text{Number of fish}}$$

$$\text{SGR } (\%/\text{day}) = \times \frac{\ln \text{ FBW} - \ln \text{ IBW}}{\text{Period (days)}}$$

where Ln = natural log

$$\text{PER} = \frac{\text{BWG (g)}}{\text{Protein intake (g)}}$$

$$\mathrm{FCR} = \frac{\mathrm{TFI\ (g)}}{\mathrm{BWG\ (g)}}$$

Sampling

The fish underwent a 24-h fasting period following a 10-week trial before blood samples were gathered. Randomly chosen three fish per replicate were sedated with a 100 mg/L benzocaine solution (Neiffer and Stamper 2009). To get blood samples, the caudal blood vessels were pierced by using plastic syringes (1 mL) without the use of anticoagulants. These samples were then centrifuged at 1198 $\times g$ for 10 min and kept at −20 °C until subsequent biochemical and immunological tests. Intestinal and liver samples (three fish/replicate) were collected for investigations of the digestive enzymes and antioxidant enzyme activity. Moreover, tissue samples from the intestine (anterior portions) were obtained and preserved in 10% buffered formalin for the intestinal histomorphometric analysis.

Intestinal digestive enzymes and biochemical assays

A whole intestine was weighed and blended in a tissue homogenizer set in an ice bath supplied with a cold saline (10 volumes; v/w) following a previous approach (Sáenz de Rodrigáñez et al. 2009). Next, the extract was centrifuged for 10 min at 13,000 ×g at 4 °C for 3 min. Following that, the supernatant was put into microtubes filled with ice. The total protein content of each sample was calculated to estimate the enzyme activity per gram of protein (Kruger 2009). Employing methods described by Bernfeld (1995) and Worthington (1993), the activities of amylase and lipase enzymes were assessed.

By following the manufacturer's instructions (MyBioSource Co., San Diego, CA, USA), the fish leptin hormone (LEP; Cat. No. MBS021271) and growth hormone (GH; Cat. No. MBS044656) ELISA kits were used to measure the serum LEP and GH levels. Furthermore, serum glucose (GLU; Ref: MD41011) and cholesterol (Ref: MD41023) levels were assayed using commercial kits (Spinreact Co., Santa Coloma, Spain) following prior proposed methods (Trinder 1969; Allain et al. 1974).

Serum immunological and hepatic antioxidant assays

Assessment of the immunological indicators including serum bactericidal activity (SBA), myeloperoxidase activity (MPO), and complement 3 (C3) were performed. Using the Liu et al. (2017) and Wangkaghart et al. (2021) approaches with minor alterations, SBA was measured against *S. agalactiae* and displayed as serum bactericidal %. After 12 h of culture in brain heart infusion (BHI) broth (Oxoid, England) at 30 °C, *S. agalactiae* was collected, reconstituted in phosphate buffer saline (PBS), and diluted to 10^2 CFU/mL. The bacterial solution (50 µL) was combined with serum samples (50 µL). Using a BHI medium rather than serum as an additional control examined the growth of bacteria in the absence of treatment activity. The mixture was plated onto BHI agar and incubated at 37 °C for 24 h. After that, the number of colonies was counted. The assay was performed in triplicate. The following equation was used to calculate SBA (%):

$$\text{SBA}\ (\%) = 100 \times \frac{\text{Number of colonies in treatment samples}}{\text{Number of colonies in control samples (BHI)}}$$

MPO activity was assessed using the previously established technique (Quade and Roth 1997). Using 3,3′,5,5′-tetramethylbenzidine hydrochloride (TMB) as a reactive peroxidase substrate, the approach is based on MPO-hydrogen peroxide (H_2O_2) oxidation. In short, serum samples (15 µL) were added to the wells of a microtitre plate and diluted with 135 µL of Hanks balanced salt solution. Following that, 50 µL of newly made substrate buffer (5 mM H_2O_2 and 20 mM TMB) was added. The color change reaction ceased after 2 min by adding 4 M sulfuric acid (50 µL). The optical density was measured at 450 nm against a blank in a microplate reader.

Using Cusabio kits (Houston, USA) and following the manufacturer's instructions, the serum level of C3 (Cat. No. CSB-E09727s) was measured. In brief, the serum samples or standards were added to each well. In the wells of serum, the horseradish peroxidase conjugate (50 µL) was applied and followed by the addition of 50 µl of antibody. After thoroughly mixing, incubation at 37 °C for 1 h was done. Washing by adding 200 µl of wash buffer was performed after aspirating each well for 10 s. The washing was repeated three times. Then, the plate was inverted and blotted with fresh paper towels. In each well, substrate A (50 µl) and substrate B (50 µl) were refilled and thoroughly mixed, followed by incubation at 37 °C for 15 min. To ensure complete mixing, a stop solution (50 µL) was included in each well. Using a microplate reader set to 450 nm, optical density was ascertained.

For estimation of hepatic antioxidant enzyme capacity involving catalase (CAT) and glutathione peroxidase (GPx), the liver samples were used; meanwhile, the total antioxidant capacity (TAC) was measured in the serum samples. The liver tissues were homogenized in PBS (pH 7.5) after being rinsed in a cold sodium chloride solution (0.9%, three times). Following that, cold centrifugation for 15 min at 1198 ×*g* was performed on the homogenates. According to Fernandez-Botran et al. (2002), the supernatants were meticulously collected in a clean tube to evaluate antioxidant indicators. The CAT (Cat. No. CA 2517), GPx (Cat. No. GP 2524), and TAC (Cat. No. TA 2513) were quantified using commercial kits from a Biodiagnostics Co. (Cairo, Egypt) via spectrophotometry.

Following the protocol of Aebi (1984), CAT activity was evaluated. The enzymatic reaction mixture utilized in this experiment consisted of homogenate supernatant, H_2O_2, 1 mL/L triton X100, 1 mM ethylene diamine tetra acetic acid (EDTA), and potassium phosphate (50 mM) at pH 7.4. The attenuation coefficient of H_2O_2 at 510 nm was measured with a UV-Vis spectrophotometer. Measurement of CAT activity was done using the quantity of CAT that degrades H_2O_2.

The GPx activity was assessed colorimetrically using the Paglia and Valentine (1967) technique at an absorbance rate of 340 nm. Organic peroxide can be converted by the GPx enzyme to an oxidized glutathione, which is subsequently recycled to its reduced state when glutathione reductase is present. The buffer constituents were 50 mM phosphate buffer, 5 mM EDTA, and 1 mM 2-mercaptoethanol at pH 7.0.

Also, TAC was measured following the prior method (Koracevic et al. 2001). Determination of TAC based on the reaction of the antioxidants in the sample with an exogenously administered H_2O_2. Residual H_2O_2 at 505 nm can be measured calorimetrically using an enzymatic reaction that facilitates the conversion of 3,5-dichloro-2-hydroxybenzenesulfonate to a colorful product.

Intestinal histomorphometric analysis

The tissue samples (anterior parts) were handled following conventional histology procedures and stained with hematoxylin and eosin (Suvarna et al. 2018). The morphometric measurements, slide inspections, and photos were done under a microscope (Ceti, England) that was outfitted with a digital camera (AmScope). To conduct the intestinal histomorphology assay, villous length (VL), villous width (VW), muscular coat thickness (MCT), and goblet cell count were evaluated (Abdel Rahman et al. 2022) using ImageJ software version 1.45 (NIH, USA).

Challenge assay

At the Department of Aquatic Animal Medicine, Faculty of Veterinary Medicine, Zagazig University, the pathogenic isolates of *C. albicans* and *S. agalactiae* strain were previously recovered from diseased *O. niloticus* and verified. Prior research by Zayed et al. (2016) and Abdel Rahman et al. (2023) determined the lethal doses of *C. albicans* and *S. agalactiae* to be 2.4×10^5 and 3.9×10^8 CFU/mL, respectively. In the challenge test, sub-lethal dosages (1.2×10^5 and 1.5×10^8 CFU/mL), respectively, were employed.

After the 10-week feeding study, the remained fish after sampling from each group were fastened for 24 h and split into three subgroups at random ($n = 11$ fish/subgroup). *C. albicans* (1.2×10^5 CFU/mL) was intraperitoneally (IP) inoculated (0.2 mL/fish) in the first subgroup. The *S. agalactiae* (1.5×10^8 CFU/mL) was IP inoculated in the second subgroup at a dose of 0.1 mL/fish. On the other hand, a sterile saline solution was used to IP inoculate the third subgroup (control). After 12 h from the inoculation, the fish were given their proper diets. For 15 days, clinical observations were maintained twice a day to document any mortalities and clinical signs. The yeast or bacteria were re-isolated from the fish organs to confirm that *C. albicans* and *S. agalactiae* were the causes of the mortalities.

Data analysis

The data's norm homogeneity was verified using Bartlett and Kolmogorov-Smirnov tests. After these analyses, all variables were subjected to an ANOVA based on polynomial orthogonal contrasts using SPSS 21.0 (IBM Corp., Armonk, USA). Furthermore, the linear and quadratic regression formulas were calculated, and $P < 0.05$ was used to indicate significance. Based on $P < 0.05$, a post-hoc Tukey's test evaluated mean variances. The means ± standard error (*SE*) of the data were displayed. Moreover, the fish survival probability in each treatment modified by *C. albicans* and *S. agalactiae* was assessed using the Kaplan-Meier model, and any significant differences between groups were explored using the log-rank test.

Table 2 The survival rate (%) and growth variables of *O. niloticus* fed dietary *M. parviflora* leaves powder (MPLP) for 10 weeks

Parameters	MPLP0	MPLP5	MPLP10	MPLP15	Linear Reg.[#]	Quadratic Reg.[#]
SR (%)	100	100	100	100	-	-
BWG (g)	41.89 ± 1.30^{d}	76.35 ± 1.61^{c}	88.19 ± 3.04^{b}	103.90 ± 1.76^{a}	< 0.01	< 0.01
SGR (%/day)	1.07 ± 0.04^{d}	1.59 ± 0.01^{c}	1.72 ± 0.04^{b}	1.89 ± 0.01^{a}	< 0.01	< 0.01
TFI (g/fish)	99.45 ± 0.42	100.27 ± 0.40	101.01 ± 0.82	101.56 ± 1.19	0.08	0.87
PER	1.31 ± 0.04^{d}	2.33 ± 0.04^{c}	2.68 ± 0.07^{b}	3.12 ± 0.08^{a}	< 0.01	< 0.01
FCR	2.38 ± 0.08^{a}	1.31 ± 0.02^{b}	1.15 ± 0.32^{c}	0.98 ± 0.03^{d}	< 0.01	< 0.01

MPLP0 (control group) = fish-fed basal diet without MPLP incorporation. MPLP5, MPLP10, and MPLP15 = fish-fed basal diets incorporated with 5, 10, and 15 g/kg MPLP, respectively. Values (means ± *SE*) in the same row with distinct superscripts showed substantial variations

SR survival rate, *BWG* body weight gain, *SGR* specific growth rate, *TFI* total feed intake, *PER* protein efficiency ratio, *FCR* feed conversion ratio

[#]The regressions were deemed significant at $P < 0.05$

Results

Survival rate and growth/feed utilization metrics

Throughout the experiment, the fish accepted every tested diet. The fish survival rate was unaffected by the dietary MPLP, and it was 100% in all groups. Table 2 demonstrates that growth and feed utilization variables (BWG, SGR%, and PER) increased linearly and quadratically ($P < 0.01$) when MPLP was incorporated into the diet compared to the control group (MPLP0). The maximum values were observed in the MPLP15 diet. There were linear and quadratic ($P < 0.01$) reductions in the FCR value of the MPLP groups relative to the control in a level-dependent way. However, MPLP diets did not alter TFI among groups.

Biochemical indices

Higher linear and quadratic ($P < 0.01$) elevations in the intestinal digestive enzymes (amylase and lipase) and GH level and a decline in LEP level by MPLP dietary incorporation (5–15 g/kg diet), with the MPLP15 group recording the highest values (Table 3). Meanwhile, all MPLP-fed fish had substantially lower GLU and cholesterol levels (linear, $P < 0.01$) in a level-dependent manner, as shown in Table 3.

Immunological and antioxidant indices

As seen in Fig. 1, the immune response was boosted by MPLP addition in a level-dependent way in terms of SBA% (Fig. 1A), MPO activity (Fig. 1B), and C3 (Fig. 1C) level, where the MPLP15 group revealed the highest values. The rise for SBA% and C3

Table 3 The biochemical variables of *O. niloticus* fed dietary*M. parviflora* leaves powder (MPLP) for 10 weeks

Parameters	MPLP0	MPLP5	MPLP10	MPLP15	Linear Reg.[#]	Quadratic Reg.[#]
Amylase (U/g protein)	3.06 ± 0.04^{d}	5.31 ± 0.19^{c}	14.30 ± 0.75^{b}	29.10 ± 0.52^{a}	< 0.01	< 0.01
Lipase (U/g protein)	0.53 ± 0.02^{c}	1.37 ± 0.07^{b}	1.84 ± 0.02^{b}	2.33 ± 0.19^{a}	< 0.01	< 0.01
LEP (ng/mL)	2.68 ± 0.18^{a}	1.63 ± 0.03^{b}	1.14 ± 0.01^{c}	0.79 ± 0.06^{d}	< 0.01	< 0.01
GH (pg/mL)	0.87 ± 0.04^{d}	2.18 ± 0.11^{c}	4.07 ± 0.04^{b}	8.61 ± 0.22^{a}	< 0.01	< 0.01
GLU (mg/dL)	66.35 ± 1.93^{a}	54.50 ± 1.44^{b}	50.80 ± 0.46^{c}	42.53 ± 0.87^{d}	< 0.01	0.21
Cholesterol (mg/dL)	132.20 ± 1.27^{a}	126.05 ± 1.12^{b}	118.35 ± 0.78^{c}	111.50 ± 0.86^{d}	< 0.01	0.74

MPLP0 (control group) = fish-fed basal diet without MPLP incorporation. MPLP5, MPLP10, and MPLP15 = fish-fed basal diets incorporated with 5, 10, and 15 g/kg MPLP, respectively. Values (means ± *SE*) in the same row with distinct superscripts showed substantial variations

LEP leptin hormone, *GH* growth hormone, *GLU* glucose

[#]The regressions were deemed significant at $P < 0.05$

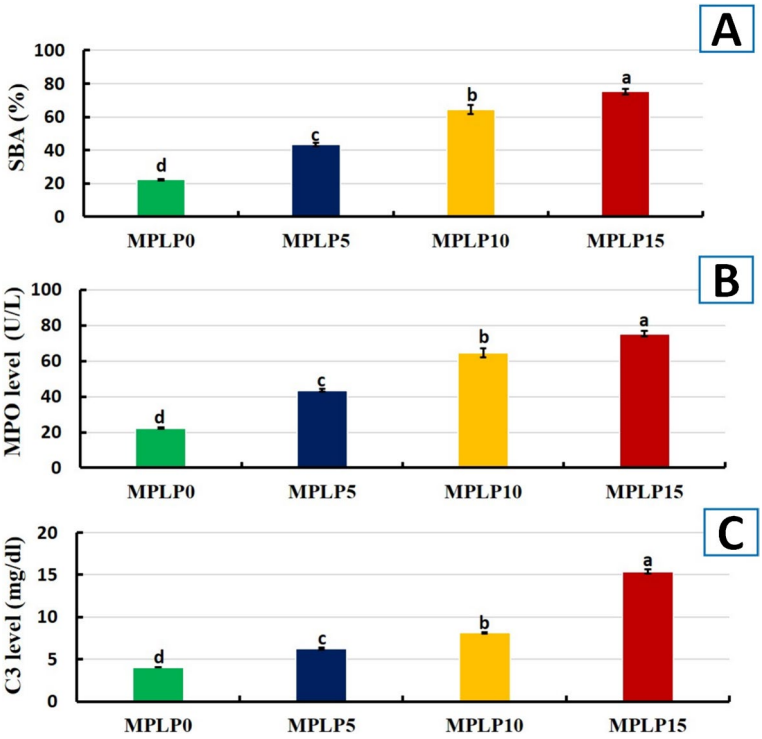

Fig. 1 The immunological variables of *O. niloticus* fed dietary *M. parviflora* leaves powder (MPLP) for 10 weeks. **A** Serum bactericidal activity (SBA %). **B** Myeloperoxidase activity (MPO). **C** Complement 3 (C3). MPLP0 (control group) = fish-fed basal diet without MPLP incorporation. MPLP5, MPLP10, and MPLP15 = fish-fed basal diets incorporated with 5, 10, and 15 g/kg MPLP, respectively. The bars (means ± *SE*) with distinct superscripts showed substantial variations ($P < 0.05$)

was linear and quadratic ($P < 0.01$), while the increase for MPO levels was linear only ($P < 0.01$).

In all MPLP-supplemented groups, the values of CAT (Fig. 2A), GPx (Fig. 2B), and TAC (Fig. 2C) elevated in a level-dependent way [linearly ($P < 0.01$) and quadratically ($P = 0.02$ for CAT, $P < 0.01$ for GPx and TAC)].

Intestinal histomorphology

Table 4 and Fig. 3(A–D) demonstrate a significant variation between groups concerning intestinal histomorphometric measures (VL, VW, MCT, and GCC) without any observed pathological changes. In all MPLP groups, there was a level-dependent marked elevation in the VL, VW, MCT, and GCC with respect to the control group (MPLP0). This elevation was linear and quadratic ($P < 0.01$).

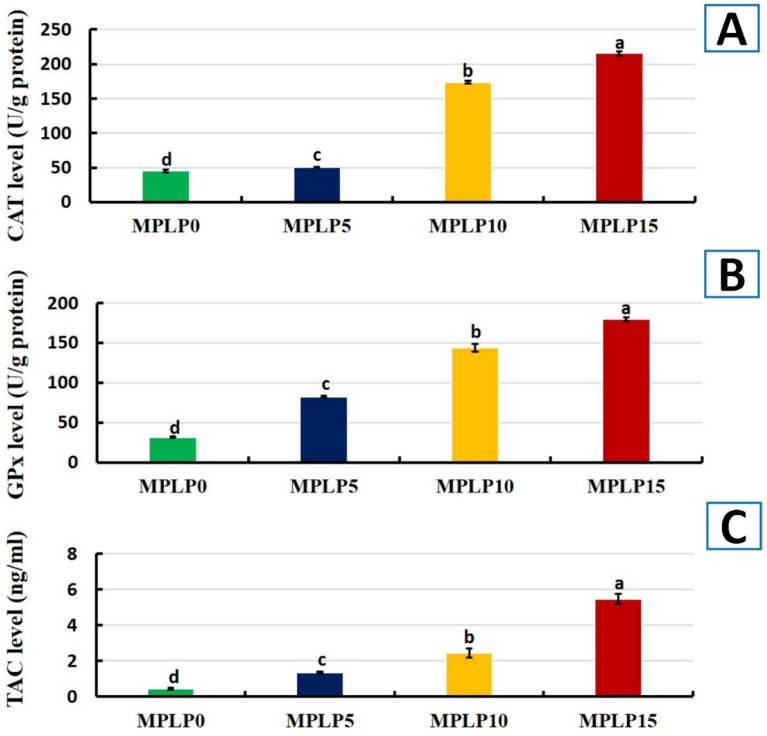

Fig. 2 The antioxidant variables of *O. niloticus* fed dietary *Malva parviflora* leaves powder (MPLP) for 10 weeks. **A** Hepatic catalase (CAT). **B** Hepatic glutathione peroxidase (GPx). **C** Serum total antioxidant activity (TAC). MPLP0 (control group) = fish-fed basal diet without MPLP incorporation. MPLP5, MPLP10, and MPLP15 = fish-fed basal diets incorporated with 5, 10, and 15 g/kg MPLP, respectively. The bars (means ± *SE*) with distinct superscripts showed substantial variations ($P < 0.05$)

Table 4 The intestinal histomorphometric variables of *O. niloticus* fed dietary*M. parviflora* leaves powder (MPLP) for 10 weeks

Parameters	MPLP0	MPLP5	MPLP10	MPLP15	Linear Reg.[#]	Quadratic Reg.[#]
VL (µm)	270.50 ± 7.79^{d}	311.00 ± 2.88^{c}	453.33 ± 3.93^{b}	693.00 ± 3.52^{a}	< 0.01	< 0.01
VW (µm)	80.50 ± 2.59^{d}	99.50 ± 2.29^{c}	107.50 ± 1.28^{b}	117.00 ± 3.57^{a}	< 0.01	< 0.01
MCT (µm)	90.39 ± 4.48^{d}	112.50 ± 1.67^{c}	127.99 ± 1.34^{b}	183.88 ± 3.30^{a}	< 0.01	< 0.01
GCC	7.67 ± 0.88^{d}	15.00 ± 1.53^{c}	23.67 ± 0.89^{b}	35.33 ± 1.45^{a}	< 0.01	< 0.01

MPLP0 (control group) = fish-fed basal diet without MPLP incorporation. MPLP5, MPLP10, and MPLP15 = fish-fed basal diets incorporated with 5, 10, and 15 g/kg MPLP, respectively. Values (means ± *SE*) in the same row with distinct superscripts showed substantial variations

VL villus length, *VW* villus width, *MCT* muscular coat thickness, *GCC* goblet cell count

[#]The regressions were deemed significant at $P < 0.05$

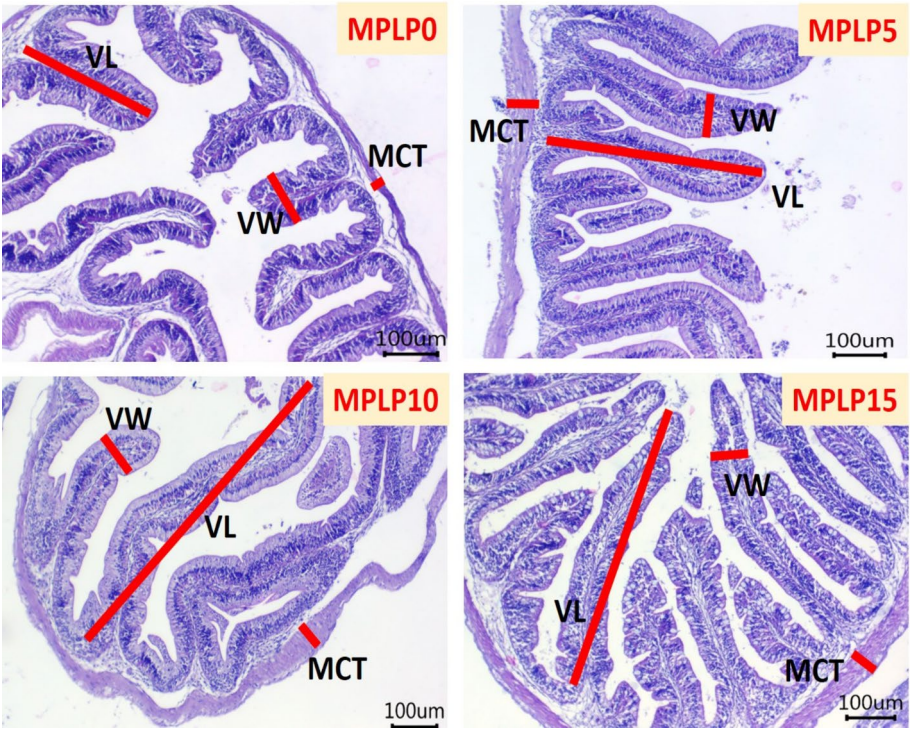

Fig. 3 Photomicrographs (H&E) of the anterior intestinal part (histomorphology) of *O. niloticus* fed dietary *M. parviflora* leaves powder (MPLP) for 10 weeks regarding villous length (VL), villous width (VW), and muscular coat thickness (MCT). MPLP0 (control group) = fish-fed basal diet without MPLP incorporation. MPLP5, MPLP10, and MPLP15 = fish-fed basal diets incorporated with 5, 10, and 15 g/kg MPLP, respectively. Scale bar, 100 µm

Resistance against *C. albicans* and *S. agalactiae* challenge

Figure 4 illustrates the survival curves (Kaplan-Meier) for *O. niloticus* challenged with *C. albicans* (Fig. 4A) and *S. agalactiae* (Fig. 4B), where the survival rate improved with increasing the dietary MPLP level. The survival rate was 63.64, 72.73, 81.82, and 90.91% in the MPLP0, MPLP5, MPLP10, and MPLP15 groups challenged with *C. albicans*, respectively. In the meantime, it was 54.54, 63.64, 81.82, and 81.82% in the MPLP0, MPLP5, MPLP10, and MPLP15 groups challenged with *S. agalactiae*, respectively. In addition to that, there was no statistically significant variations were seen among groups.

Discussion

Maintaining exact fish growth conditions is the main emphasis of the aquaculture industry nowadays. This entails that producing advantageous aquafeeds with substantial food components and useful additives is crucial (Ahmadifar et al. 2021). Food additives

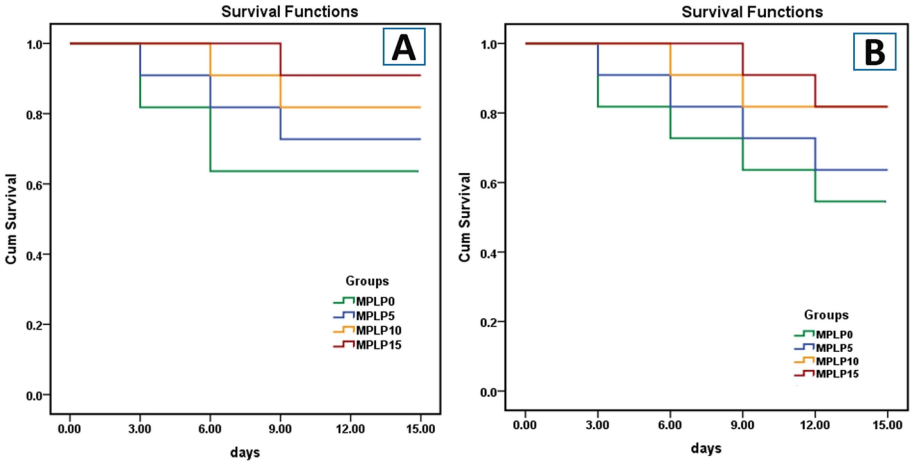

Fig. 4 Survival curves of *O. niloticus* fed *M. parviflora* leaves powder (MPLP) for 10 weeks and after that challenged with *C. albicans* **A** and *S. agalactiae* **B** for 15 days. MPLP0 (control group) = fish-fed basal diet without MPLP incorporation. MPLP5, MPLP10, and MPLP15 = fish-fed basal diets incorporated with 5, 10, and 15 g/kg MPLP, respectively

in aquafeeds, especially herbal components can improve the rate of absorption in the digestive tract and increase survival (Dawood et al. 2022). Therefore, the purpose of this work was to examine the potential beneficial impact of MPLP as a feed additive on the overall health status of *O. niloticus*.

Monitoring fish growth characteristics in response to dietary natural additives is a useful method for assessing fish health. Fish growth is closely correlated with its capacity for nutrition, absorption, and digestion (Brosset et al. 2023; Gaffar et al. 2023). According to the study's findings, the growth metrics of *O. niloticus* were enhanced by dietary MPLP supplementation (5–15 g/kg). The rationale for this outcome may be that MPLP diets increased GH levels as seen in our results. The somatotropic cells of the pituitary gland secrete GH, which is essential for animal growth and the elevated GH level supports this finding (Velloso 2008). Additionally, pituitary GH regulates the synthesis of LEP, and the increasing GH levels reduce the production of LEP in fish (Douros et al. 2017), this was confirmed in our results.

An additional plausible rationale for the enhanced growth in our study is raising the intestinal digestive enzyme activity, including amylase and lipase in all MPLP groups reflecting enhanced digestion. By breaking down starch and glycogen, amylase plays a crucial part in the digestion of fish (Nolasco-Soria 2021). For the intestine to absorb lipids, fish have a lipase enzyme that breaks down fat (Zhang et al. 2020). Thus, the activation of the enzymes' activity enhanced digestion (Magouz et al. 2020; Abdel Rahman et al. 2021). Since the fish intestine is the principal site of nutritional absorption, its histological properties are extremely valuable for investigations on nutrition (Amer et al. 2022). In this study, it was found that dietary supplementation of MPLP preserved gut structure and improved gut morphometric measurements (VL, VW, MCT, and GCC) in a level-dependent manner. These findings showed an increased porous surface area and improved integrity of the intestinal brush barrier. Furthermore, they enhanced the immune response against pathogens that invaded the intestinal mucosa by forming a viscous mucus. All these outcomes showed that

appropriate MPLP as a dietary supplement can enhance the fish's capacity for digestion and encourage the absorption of nutrients, which increases growth. Comparable findings have been described for gilthead sea bream (*Sparus aurata*) and European sea bass (*Dicentrarchus labrax*) (Bilen et al. 2019) and rainbow trout (*Oncorhynchus mykiss*) (Rashidian et al. 2020).

Blood GLU acts as the main energy source for fish to endure adverse conditions and is a reliable stress indicator (Malini et al. 2018; El-Dakar et al. 2023). The lipids found in bloodstreams as lipoproteins are cholesterol. The diet constituents are the most significant factor among the various that affect the concentration of cholesterol (Kondera et al. 2017). In the groups receiving MPLP supplements, the blood GLU and cholesterol decreased; the MPLP15 group had the lowest values. These findings mean that MPLP supplementation increased the utilization of carbohydrates that beneficially impacted the fish's metabolism. The oleanolic acid of *M. parviflora* demonstrated hypoglycemic and hypolipidemic properties as well as improved insulin resistance (Gutiérrez 2017). Additionally, gallic acid is highly concentrated in the total phenolics evaluated in *M. parviflora* and plays a major role in decreasing hyperglycemia and hyperlipidemia (Mohammed et al. 2022). This outcome is consistent with earlier research (Rashidian et al. 2020) that demonstrated a reduction in the blood GLU of *O. mykiss* provided *Malva sylvestris* diets.

The primary barrier against any infection is non-specific immunity. Functional ingredients found in fish diets may modulate the immune system (Sahoo et al. 2021; Amer et al. 2024). According to our research, the immune variables (SBA%, MPO, and C3) were raised in response to increasing the MPLP supplementation level. These results validated MPLP's immunostimulant potential which leads to better growth. These outcomes stem from different components of flavonoids found in *Malva* that directly modulate the immunological components (Yang et al. 2012; Saad et al. 2017). Moreover, daucosterol, a component of *M. parviflora*, can stimulate macrophages according to Ramírez-Serrano et al. (2019). Parallel to this, Afifi (2016), Bilen et al. (2019), and Bilen et al. (2020) reported that the immune responses of *O. niloticus*, *S. aurata*, *D. labrax*, and *O. mykiss* (respiratory burst, MPO, lysozyme, and phagocytic activity) increased in response to varying dietary *M. sylvestris* supplementation.

Oxidative stress arises from a mismatch between the production of reactive oxygen species and the antioxidant defense system (Biller and Takahashi 2018). The present study revealed that dietary MPLP increased antioxidant response (CAT, GPx, and TAC). The polyphenols, malvin, malvaline, scopoletin, sterols, and vitamins (E and C) of MPLP with their antioxidant properties may account for these results. These compounds can scavenge a variety of free radicals, protecting tissues from oxidation (Bouriche et al. 2011; Dugani et al. 2016; Lagunas-Herrera et al. 2019). According to prior research (Adam et al. 2018), M. *parviflora* leaves have the strongest antioxidant activity without inducing any cytotoxicity. A previous study (Khoris and EL-Sherbeny 2022) corroborated these results.

A challenge assay is employed to evaluate the fish's immune system. Our investigation demonstrated that dietary MPLP protected fish from *C. albicans* and *S. agalactiae* infection by increasing their survival rates. The highest survival rates were noted in response to the MPLP15 diet. The positive impacts of MPLP on fish survival can be attributed to its potent antioxidant and immunological properties (as illustrated above). The direct antifungal and antibacterial activity of *M. parviflora* was reported in vitro (Shadid et al. 2021; Mohammed et al. 2022). Furthermore, dietary *Malva* spp. enhanced the resistance of different fish

species to *Vibrio anguillarum*, *Yersinia ruckeri*, and *Pseudomonas fluorescens* (Bilen et al. 2019; Rashidian et al. 2020; Khoris and EL-Sherbeny 2022).

Ultimately, MPLP diets can be added to the fish feed to enhance the health and growth of *O. niloticus*, assisting in the aquaculture industry's continued sustainable growth.

Conclusion

In this work, we evaluate the impact of dietary MPLP supplementation on the growth and health status of *O. niloticus*. Based on the aforementioned data, it can be concluded that adding MPLP to *O. niloticus* diets improved the organisms' growth, digestive/absorptive capacities, and antioxidant-immuno potentials. Remarkably, it also increases fish resistance to *C. albicans* and *S. agalactiae*, enabling its use as a natural antifungal and antibacterial agent. It is still necessary to research the promising effects of MPLP in other fish species' diets including its other antimicrobial potentials and its protective impact on different water pollutants.

Acknowledgements The authors thank the Aquatic Animal Medicine Department, Faculty of Veterinary Medicine, Zagazig University, for their help during the experimental procedures.

Author contribution A. N. A., Y. A. R., G. E., E. M. Y., A. A. A., N. A., A. A. K., A. Y., S. J. D., and R. E. I.: conceptualization, data curation, formal analysis, investigation, methodology, resources, validation, and visualization; A. N. A.: writing—original draft; A. N. A. and R. E. I.: writing—review and editing

Funding This work was supported by the Researchers Supporting Project (RSP2024R36), King Saud University, Riyadh, Saudi Arabia.

Data availability All data generated or analyzed during this study are included in this article.

Declarations

Ethics approval The Institutional Animal Care and Use Committee of Zagazig University in Egypt accepted the experimental protocol (ZUIACUC–2-F–2–2024).

Competing interests The authors declare no competing interests.

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