Assiut University web-site: www.aun.edu.eg

TRYING TO USE ANTIBIOTICS ALTERNATIVES TO RAISE IMMUNE EFFICIENCY AND GROWTH PERFORMANCE IN *TILAPIA NILOTICA*

ENAS, A.I. KHORIS[1] AND EMAN M.E. EL-SHERBENY[2*]

[1] Fish Disease Unit, Animal Health Research Institute (AHRI), Tanta lab., Agricultural Research Center (ARC), Giza, Egypt
[2] Pharmacology Unit, Animal Health Research Institute (AHRI), Tanta lab., Agricultural Research Center (ARC), Giza, Egypt

Received: 16 November 2021; **Accepted:** 10 January 2022

ABSTRACT

This study examined *in vitro*, the antibacterial activity of *Malva parviflora* leaves ethanolic extract against *Pseudomonas fluorescens* and its minimum inhibitory and minimum bactericidal-concentrations (MIC, MBC), identified its biological compounds responsible for its efficacy by Gas Chromatography-Mass Spectrometer (GC-MS) device besides evaluated its effect *in vivo* on Nile tilapia fish as a medicinal plant to combat *P. fluorescens* challenge. Two hundred twenty-five fishes were divided into five groups; G1: negative control. G2: positive control; infected at the 60 days (1x103CFU/0.2ml I/M). G3, G4 and G5 were fed with (0.25, 0.5 and 1 g/kg) respectively (60 days), then infected. On the thirty, sixty days and post challenge, blood samples were collected. Sensitivity test revealed the susceptibility of *P. fluorescens* to the extract; MIC and MBC were 3.90 and 31.25 mg/ml respectively. Body weight, phagocyte nitric oxide and lysozyme activity increased significantly in all treated groups than controls especially G4 while both G3 and G4 increased the survival rate to 80% compared to G2 (40%). Total leucocytic, lymphocyte (L) and monocyte (M) counts increased significantly in all treated groups than G1 throughout the study. Aspartate (AST) and alanine aminotransferases (ALT), alkaline phosphatase (ALP), urea and creatinine showed no significant difference between G3 and G1, while G4 and G5 increased in a dose-dependent manner. Total protein (TP), albumin and globulin revealed no significant difference among groups. Malondialdehyde (MDA) showed the best decrease in G3 than G1. Post challenge all treated groups elicited a significant decrease than G2. Superoxide dismutase (SOD) and reduced glutathione (GSH) were significantly higher in all treated groups than the controls in varying degrees. Finally, the used extract enhanced the fish performance, immune system and antioxidant biomarkers without any side effects on liver and kidney functions at 0.25 g/kg, while 0.5 g/kg gave the highest weight gain.

Key words: *Malva parviflora* ethanolic-extract; *Pseudomonas fluorescens*; *Nile tilapia*; medicinal-plant; GC-MS

Corresponding author: EMAN M.E.EL-SHERBENY
E-mail address: myvetaddress@gmail.com
Present address: Pharmacology Unit, Animal Health Research Institute (AHRI), Tanta Lab., Agricultural Research Center (ARC), Giza, Egypt

INTRODUCTION

Bacterial diseases considered as one of the most prominent obstacles in fish culture lead to huge economic losses if not controlled and prevented in advance. Pseudomonas is a stress related infection that considers the most common opportunistic bacterial infection in aquaculture especially among fresh water fish. While *Pseudomonas fluorescence* is a natural microbiota of fish gut and the aquatic environment, it considers one of the most recurrently identified epizootic risk aspects affecting fish worldwide. The pathogenic strains of *P. fluorescens* characterized by hemolytic and proteolytic possessions, and they speedily gain resistance against commonly used antibiotics (Terech-Majewska, 2016). This pathogen leads to a grave warning for water pollution sensitive fish (Golas *et al.*, 2019). The rapid identification and diagnosis of fish microbes in aquaculture is needed for successful disease manage and treatment (Raman, 2017).

Although the application of antimicrobial compounds can be useful and effective in treatment of pathogens in fish farms, the residues of these compounds in fish musculature is harmful to consumers (Diler *et al.*, 2017). Furthermore, the frequent using and tolerance to the existing drugs in fish culture initiates antibiotic resistant strains of bacteria and decreases their efficacy (Capita and Alonso-Calleja, 2013). So, the alternatives to antibiotics which enhance a similar protection to aquatic animals are urgently needed. One of these promising alternative strategies is medicinal plant that combat microbial diseases and become the focus of several studies (Terzi and Isler, 2019).

Herbal plants have been identified for traditional use in caring for several human diseases since a long time ago and now they considered in aquaculture to give safe composites which can be used as suitable substitutes to synthetic antimicrobial compounds. Various extracts of the leaves, flowers, seeds, and roots of these plants have been applied in Tilapia aqua farm and administered to fish through immersion or orally in fish ration. Its biological activities include promotion of performance and feed nutrients utilization, stimulation both of the cellular and humoral immunity and gene expression consequently, increase fish confrontation against diseases (Kuebutornye and Abarike, 2020). Application of plant origin treatment as a chemotherapeutic agent in fish field is a relatively new direction of research. These plants can be used as fresh, dried powder, essential oils, or extracted (by different solvents like water, alcohol, acetone, etc). Medicinal plants have various actions like growth promotion, antimicrobial and antioxidant activities plus their anti-stress potentialities (Reverter *et al.*, 2014). Numerous active constituents such as alkaloid, pure volatile oils, terpenoids, essential oils, lipopolysaccharides, saponins, phenolics, polypeptides, flavonoids and several other compounds of plant origin are chemotherapeutic agents, that have antibacterial activity, so can be considered as an alternative to antibiotics (Saxena *et al.*, 2013).

Malva parviflora or little mallow is one of the medicinal plants with a pharmacological potential in combating different ailments in aquaculture. It is found in North Africa and widely found in Europe. It has several common names like Egyptian mallow under local name of Khabazi. It is an annual or perennial herb, flowers and leaves are the most commonly used parts of the plant, since leaves consider the richest part in phenols and flavonoids. It has a promising pharmacological effect as analgesic, antibacterial, antifungal, anti-irritant, skin treatment (antiseptic, emollient, demulcent), antiulcerogenic, anti-inflammatory and antipyretic activity (Singh and Navneet, 2017). Its active ingredients include mucilage, tannins, anthocyanines, ascorbic acid, naphtaqiunone, malvyn, terpenoids,

malvidin and volatile oils (Gasparetto *et al.*, 2011). *M. parviflora* leaves are well off nutraceuticals such as antioxidants; (phenol derivatives, tocopherols, carotenoids, along with flavonoids), minerals, and unsaturated fatty acids as α linolenic acid (Barros *et al.*, 2010; Beghdad *et al.*, 2015). Various pharmacological studies of *M. parviflora* extracts and their efficacy were reported by European Medicines Agency, (2019).

This study aimed to explore *in vitro*; the antibacterial activity of the ethanolic extract of *M. parviflora* leaves against *P. fluorescens* and its MIC and MBC values, besides detection of its active compounds using the GC-MS analysis device to provide insight into its potential constituents. A pilot study was performed using 3 concentrations of *M. parviflora* extract (250, 500 and 1000 mg/kg ration) to evaluate its protective efficacy and its effects *in vivo* on growth performance, survival rate, immunological responses, hematological, biochemical profiles and antioxidant status of Nile tilapia fish as a medicinal plant support the disease resistance against *P. fluorescens* challenge.

MATERIALS AND METHODS

1. Plant materials and extract preparation

M. parviflora extract was prepared and obtained from the National Research Center, Egypt according to Klūga *et al.* (2017). Fresh leaves of *M. parviflora* plant as described by Akbar *et al.* (2014) were initially dried in the shade, ground to fine particles and an amount of 10 g flooded in 100 ml of absolute ethanol (Sigma) for 48 hr with frequent agitation. Then, the ethanolic extract was filtered through the What man filter paper and the resulted filtrate was concentrated using a rotary evaporator under reduced pressure at 40°C. The dried extract was weighed for usage in antibacterial assay and the experiment.

2. Detection of active compounds of *M. parviflora* leaves ethanolic extract using Gas Chromatography-Mass Spectrometer (GC-MS)

Analysis was performed in the laboratory of the Scientific Research Center and Measurements (SRCM), Tanta University. Sample was extracted using GC Program Acquisition Parameters; instrument (Perkin Elmer model; clarus 560S). Oven; Initial temperature 50°C/4min, ramp1; 10°C/min to 150°C, hold 5 min, ramp2; 10°C/min to 280°C, hold 1 min, Volume=1 µL, Inj=280°C, Split=20:1, Delay of solvent =3 min, Carrier gas=Helium, Transfer Temperature=280°C, Source temperature =200°C, Scan; 50 to 620Da, Column; Elite 5MS; 30m 0.25mmID with 0.25um df and Sampling rate: 12.50000 pts/s. Preliminary recognition of the various constituents was performed by matching up their mass spectra to the literature (MAINLIB, Pfleger and Replib). Compounds % was computed from GC peak areas (Al-Qarawi and Al-Obaidi, 2018).

3. *In vitro* antibacterial activity tests

3.1. Antibacterial sensitivity test (AST)

The antibacterial activity of the used extract on *P. fluorescens* was assessed using disc diffusion method (Jorgensen *et al.*, 1999). Sterilized filter paper discs (6 mm) were individually saturated with 20µl of stock solutions of 4 extract concentrations (125, 250, 500 and 1000 mg/ml) and placed onto *P. fluorescens* inoculated agar then incubated at 35°C/18h. To compare the antibacterial effect of the used extract, commercial disks of Gentamicin (CN; 10 µg) and Ciprofloxacin (CIP; 5 µg) were used. The resulted inhibition zone diameters (IZD) were exacted in millimeters (mm). The test was applied in triplicate and mean values calculated.

3.2. Minimum inhibitory concentration (MIC)

The MIC assay was definited by using micro broth dilution test in a 96 well microplate using standard procedure of CLSI, (2015). A solution of 125 mg/ml concentration was two folds serially diluted till 0.25 mg/ml. Both negative and positive control wells were performed. Dimethylsulfoxide (DMSO,

Sigma) was used to indicate the presence of uninhibited bacterial growth in each well. Referring to the results of the MIC assay a loopful, from each clear well was streaked on the specific media for *P. fluorescens* incubated at 35°C/20hr, then observed for growth. MIC was the lowest concentration that showed no turbidity and minimum bactericidal concentration (MBC) was the lowest concentration did not show any growth. The experiment was performed in duplicate.

4. *In vivo* assessment of the extract

4.1. Fish and Experimental design:

Apparently healthy 225 *Oreochromis niloticus* fish have normal behavioral reflexes and free from any visible skin lesions (100.40 ± 1.55 g/fish) were attained from a private fish farm in Kafr El-Sheikh Governorate, transferred to AHRI, Tanta Lab, and kept for acclimatization for 2 weeks earlier to the experiment. Fish were placed in glass aquaria (70×40×30cm) at (26±1°C) and chlorine gas free tap water (Innes, 1966) with continuous aeration by air compressor. Fish were fed twice daily (09:00 and 16:00) with 3% of their b. wt. (Eurell *et al.*, 1978). Fish excreta were siphoned daily manually and replaced 30% of water. Fish were separated into triplicate 5 groups (15 fish/aquarium) and reared for 75 days. A pilot study was used to assess the effect of 3 doses of *M. parviflora* extract as follow: G1: negative control; fish were fed a basal commercial pellet, for 60 days then fish injected intramuscularly (I/M) with 0.1 ml of 0.9% sterile saline at the 60 days. G2: positive control; fish were fed basal ration for 60 days then fish injected I/M with ($1x10^3$CFU/0.2ml) *P. fluorescens* at the 60 day. G3, G4 and G5: fish were fed a prepared ration supplemented with 0.25, 0.5, 1 g/kg of *M. parviflora* extract respectively for 60 days then fish infected at the 60 days.

The experimentally infected fish were inspected daily post infection (PI), the clinical signs, moralities and necropsy finding were record for 15 days after infection. Re-isolation of *P. fluorescens* pathogen from kidney and liver of moribund experimentally infected fish was done to confirm that mortalities occurred due to the infection.

4.2. Pseudomonas fluorescens and challenge test:

P. fluorescence was obtained from Poultry and Fish Diseases Department, Fac. Vet. Med. Alex. Univ. Egypt. After 60 days of feeding, infected groups were injected I/M with ($1x10^3$CFU/0.2ml) *P. fluorescence* (Khalil *et al.*, 2010). Mortalities and survival fish were observed for15 days after infection and results were recorded.

4.3. Growth parameters and survival rate calculations

Weight of fish at 0, 30 and 60 day was recorded. Parameters were calculated as follow; specific growth rate (SGR) = 100 × [(Ln final fish wt) − (Ln initial fish wt.)]/days fed. Weight gain (%) = (final fish wt initial fish wt)/initial fish wt × 100. Feed conversion ratio (FCR) = feed intake (g)/wt gain (g). Fish survival % = 100 × final number/initial number.

4.4. Blood samples:

Three types of samples were taken 3 times from the caudal vein (5 fish/group) on 30th, 60th days and post-challenge (PC) at the end of the experiment. According to Stoskopf, (1993) 1st sample was taken on EDTA tube for hematological investigation. The total leucocytic count (T.L.C) was determined manually using the hemocytometer (Blaxhall and Daisley, 1973). The differential leukocyte count (D.L.C) was performed using Wright Giemsa-stained blood film. Percent of each type of cells was recorded (Blaxhall, 1972). The 2nd sample was taken in clean dry centrifuge tube without anticoagulant for serum separation for biochemical investigations: aspartate and alanine aminotransferases (AST and ALT) activities (Reitman and Frankel, 1957), Alkaline phosphatase (ALP) (Tietz, 1995), total protein (TP) (Doumas *et al.*, 1981),

albumin (Reinhold, 1953). Globulin was detected by subtraction albumin value from TP and albumin/globulin ratio was detected by dividing both values. Malondialdehyde (MDA) (Yagi, 1984), reduced glutathione (GSH) (Beutler *et al.,* 1963), superoxide dismutase (SOD) (Nishikimi *et al.,* 1972), urea (Batton and Crouch, 1977) and creatinine (Houot, 1985) were also estimated. All testes were determined using commercial kits (Spectrum, ELITech, BioSystems and Biomed Companies, Egypt) in accordance with the manufacturer's instructions. The 3rd sample was taken in heparinized syringe for nitric oxide (NO) assay.

4.5. Determination of immunological parameters

1) Measurement of phagocytic activity of macrophage supernatant expressed as NO assay in heparinized blood using ELISA reader (Rajarman *et al.,* 1998; Municio *et al.,* 2013).

2) Estimation of lysozyme activity by agarose gel lysis assay in serum sample (Schltz, 1987).

5. Water samples

They were taken every two weeks to determine the water quality parameters as the standard guidelines of APHA, (1998). The parameters are dissolved oxygen by using digital meter (HI 9142, HANNA, China), water temperature by using digital thermometer, PH measured by PH indicator strips (MColorpHast-Germany), salinity meter used for salinity measurement (YSI Eco Sense EC300 Salinity/Conductivity 151, China), kits for measuring the degree of total ammonia, unionized ammonia (NH3), nitrate (No3) and nitrite (No2) in the water (USA, Virginia Company, lot. No.201134).

6. Statistical analysis

Statistical tests were applied to determine the best treatment using one-way ANOVA Test and regard as significant when ($P<0.05$). Data analysis was carried outby SPSS 20 software as mean ± SE and (n=5) (Petrie and Watson, 1999).

RESULTS

1. Detection of active compounds of *M. parviflora* leaves extract by GC-MS

The chemical constituents' chromatogram of the extract peaks was detected using gas chromatography joined to mass spectrometry (GC-MS) (Fig. 1). In Table 1, the principal and slight components of the extract identified by GC-MS according to area % of peaks at specific retention time (RT). The major compounds (Peak 16: 45.027%) were Hexadecanoic acid, 1-(hydroxymethyl)-1,2-ethanediyl ester and Ascorbic acid 2,6-dihexadecanoate. The second most abundant compound was Oleic Acid (Peak 15: 26.338%) in addition to Octadecanoic acid, octadecyl ester / Tetradecenoic acid / E-8-Methyl9-tetradecen-1-ol acetate and Z-8-Methyl-9-tetradecenoic acid. The third major peak (Peak 14: 10.487 %) represents Triarachine and Hexadecanoic acid, 2-(octadecyloxy) ethyl ester. Another vital fatty acids, esters and other compounds constituted an important portion of the extract were also detected as Thiocyanic acid, ethyl ester, Vaccenic acid, Erucic acid, Tetradecanoic acid, Octadecanoic acid, Myristic acid, Linoleic acid, Eicosanoic acid, Palmitic acid, and Phytol.

2. *In vitro* antibacterial activity screening tests

2.1. Antibacterial sensitivity test (AST)

All of extract concentrations (125, 250, 500, 1000 mg/ml) showed antibacterial effect on *P. fluorescens* giving increased IZD; 11 ± 0.33, 12 ± 0.67, 14 ± 0.33 and 16 ± 0.40 mm respectively. MIC and MBC values were 3.90 and 62.50 mg/ml respectively. IZDs of Gentamicin and Ciprofloxacin were (17 ± 0.20 and 22 ± 0.40 mm), respectively.

3. *In vivo* assessment of the extract

3.1. Clinical signs

Groups 1 did not show any abnormal signs or mortalities during the experiment. Experimentally infected fish with *P. fluorescens* (Fig. 2 a, g) showed a typical external and internal sign similar to those caused by natural infection in the first week of the infection such as anorexia, dullness, loss of reflexes, slight abdominal distention. (Fig. 2 a, b) showing hemorrhagic patches on skin, fins, around mouth and at the base of lower jaw and head (Fig. 2 c) showing tail and fin rot, (Fig. 2 d) showing darkness in color and detachment of scales with points of hemorrhage, (Fig. 2 e) showing skin erosions and ulcerations. Moreover, P.M. lesions were slight ascites, oedema, (Fig. 2 f) showing liver paleness and enlargement with hemorrhagic spots and (Fig. 2 g) showing enlargement and congestion of kidney, spleen and gills. To confirm the causative agent of the infection, *P. fluorescens* were re-isolated from skin ulceration and lesions, liver and kidney of infected and dead fish.

3.2. Growth performance and survival rate

In Table 2, all treated groups resulted in improved growth performance and survival rates besides decreased FCR when compared with the control. G3 and G4 exhibited significantly higher final body weight, weight gain, SGR and survival rate especially G4.

3.3. Lysozyme activity and phagocyte NO level

According to Table 3, after 30, 60 days and post challenge no significant difference in lysozyme activity was recorded between G3 and G5 while the highest increase was recorded in G4. Both G4 and G5 increased significantly than controls throughout the experiment, while G3 increased than controls only post challenge. Regarding to NO level, after 30 days no significant difference was recorded between G3 and G5, values arranged in this ascending order G4, G5 then G3. After 60 days, values arranged in this ascending order G5, G4 then G3. Post challenge positive control increased significantly in NO and lysozyme levels than negative control. No significant difference was recorded between G3and G4, values arranged in this ascending order G5, G3 then G4. All treated groups showed a significant increased than both controls before and after challenge.

3.4. T.L.C and D.L.C

In Table 4, after 30, 60 days and post challenge, a significant increase in TLC, lymphocyte and monocyte counts recorded in all treated groups compared to negative control, while positive control showed a significant increase in these items post challenge only.

3.5. Serum biochemical parameters

Regarding to the activities of liver enzymes AST, ALT and ALP in Table 5, results after 30 and 60 days revealed no significant difference between G3 and G1 while, G4 and G5 showed a significant increased, values arranged in this ascending order G3, G4 then G5. Post challenge G2, G4 and G5 increased significantly in the activities of these enzymes than G1, values arranged in this ascending order G3, G4, G5 then G2. Results of TP, albumin, globulin and A/G ratio after 30 and 60 days revealed no significant difference among G3, G4 and G1 in most of these parameters while G5 showed a significant decreased. Post challenge G2 and G5 showed a significant decreased in TP, albumin, globulin than G1.

3.6. Antioxidant biomarkers and kidney function tests

Results of MDA levels in Table 6 after 30 and 60 days decreased significantly in G3 and increased in G4 and G5 than G1, while G4 and G5 did not show any significant difference between each other. Post challenge positive control showed a significant increase than negative control. All treated groups elicited a significant decrease than G2 and arranged in this descending order G5, G4 then G3. Regarding to SOD, after 30 days all treated

groups showed a significant increased than G1, values arranged in this ascending order G3, G5 then G4. After 60 days, no significant difference was recorded between G5 and G1, while G3 and G4 were significantly lower than G1. Regarding to GSH a significant difference was recorded among treated groups. After 30 days, G3 showed a significant increased than G1; values arranged in this ascending order G4, G5 then G3. After 60 days, values arranged in this ascending order G4, G3 then G5, while all of them were significantly lower than G1. Post challenge results of SOD and GSH levels showed a significant decreased in G2 than G1; also, G3 and G5 did not show any significant difference between each other. All treated groups were significantly higher than both controls.

Regarding to urea after 30 and 60 days, G4 showed no significant difference while G5 showed a significant increase compared to G1. Regarding to creatinine after 30 days, G3 and G4 showed a significant decreased than G1, while G5 showed no significant difference. After 60 days, both G4 and G5 showed a significant increased than G1 and values arranged in this ascending order G3, G4 then G5. Post challenge, G2 showed a significant increased than G1. All treated groups elicited a significant decrease than G2 and arranged in this descending order G5, G4 then G3.

4. Water quality parameters

Water parameters were: temperature 26 ±1°C, pH from (7.5 – 8), dissolved oxygen 7± 0.8 mg/L and salinity ‰ 11 to 12, total ammonia from (0.165 - 0.239) mg/L, unionized ammonia (NH3) from (0 - 0.05) mg/L., Nitrite (No2-N) from (0.07 - 0.20) mg/L and nitrate (No3-N) from (2 - 3.35) mg/L.

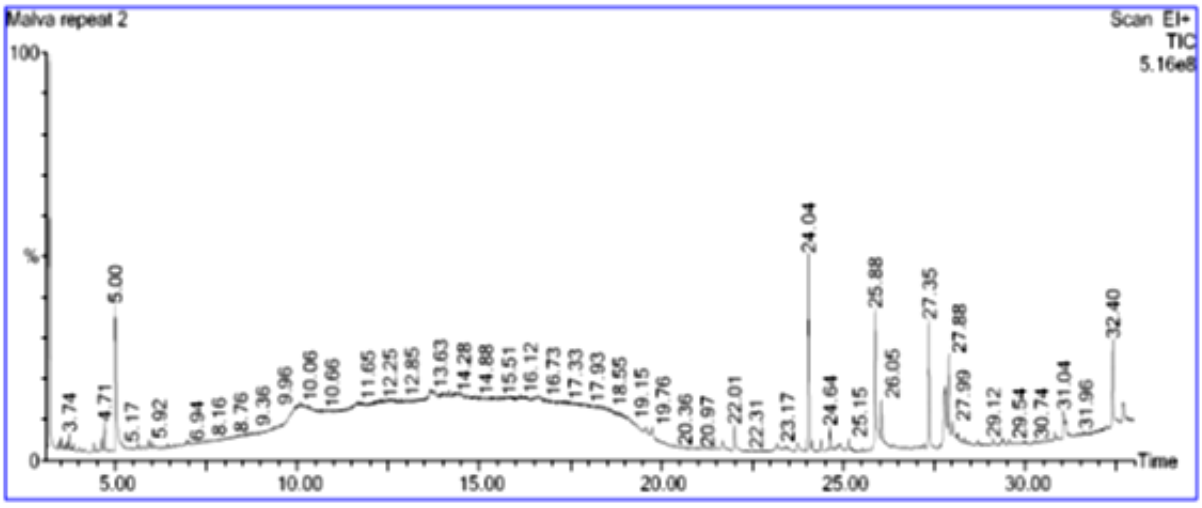

Fig. 1: Chromatogram of ethanolic extract of *M. parviflora* leaves elucidate GC-MS analysis showing various peaks with varying areas (represent many compounds in different amounts) obtained at different retention times.

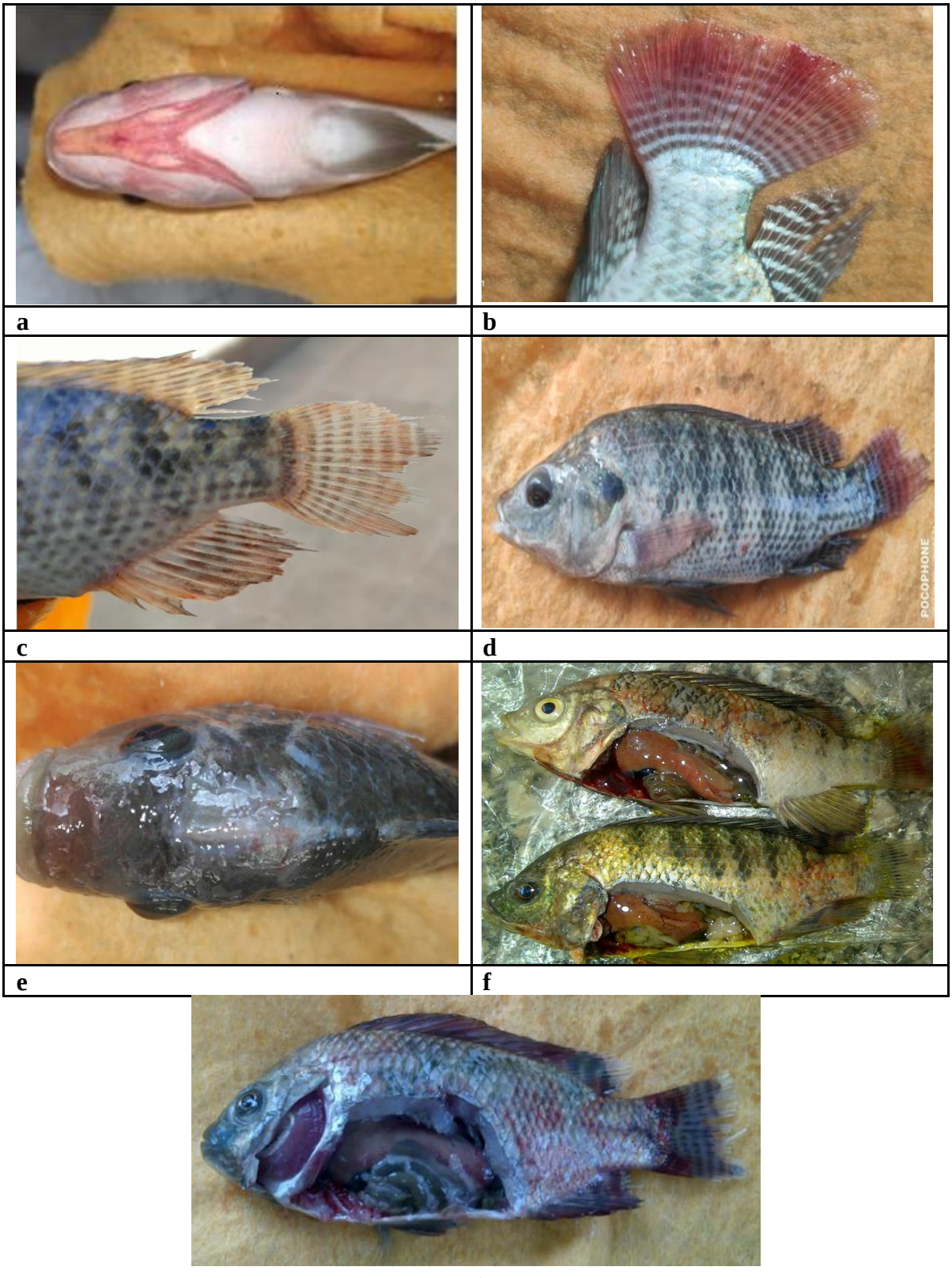

Fig. (2) Lesions of the experimentally infected *Oreochromis niloticus* fish with *P. fluorescens* a, b : showing hemorrhagic patches on skin, fins, around mouth and the base of lower jaw and head, c: showing tail, fin rot and erosion, d: showing darkness in color, tail and fin rot and erosions, detachment of scales with hemorrhagic spots and ulceration, e: showing skin erosions and ulcerations on head and trunk and hemorrhage around mouth, f: showing liver paleness and enlargement with hemorrhagic spots and g: showing tail and fin erosions, congestion of gills, internally enlargement and paleness of liver, congested kidney and spleen.

Table 1: The composition of *M. parviflora* leaves extract illuminates by GC-MS analysis.

Peak	Retention time (RT)	Compounds	Area %
1	3.198	Propane, 2,2- diethoxy- Diisopropyl ether / Thiocyanic acid, ethyl ester / Silane, triethyl-	1.854
2	3.524	Oxirane, (butoxymethyl)-	0.074
3	3.744	Pentane, 1-ethoxy-/ 2-Hexanol, 2,3-dimethyl-	0.088
4	4.419	Octane, 1-ethoxy-/ Oxirane, [[(2-ethylhexyl)oxy]methyl]-	0.061
5	4.619	Cyclohexane, 1,2-dimethyl-, cis-/ Cycloheptane, methyl-	0.095
6	4.709	Cyclohexane, ethyl-	0.167
7	5.004	2-Pentanone, 4-hydroxy-4-methyl- / 2-Hexanol, 2-methyl-	1.289
8	6.625	3-Trifluoroacetoxydodecane / 2-Undecanethiol, 2-methyl-	0.053
9	6.740	Oleic Acid	0.085
10	6.960	Z-8-Methyl-9-tetradecenoic acid	0.119
11	7.015	1-Hexadecanol, 2-methyl-/ Oxirane, [(hexadecyloxy)methyl]-	0.075
12	7.500	trans-13-Octadecenoic acid / cis-Vaccenic acid	0.499
13	8.416	Hexadecane, 1,1-bis(dodecyloxy)-	1.663
14	10.056	Oleic Acid / Triarachine / Hexadecanoic acid, 2-(octadecyloxy)ethyl ester	10.487
15	13.658	Oleic Acid / Octadecanoic acid, octadecyl ester / tetradecenoic acid / E-8-Methyl-9-tetradecen-1-ol acetate / Z-8-Methyl-9-tetradecenoic acid	26.338
16	14.168	Hexadecanoic acid, 1-(hydroxymethyl)-1,2-ethanediyl ester Ascorbic acid 2,6-dihexadecanoate	45.027
17	19.575	Cyclopentadecanone, 2-hydroxy-/ 7-Hexadecenal, (Z)-	0.639
18	19.755	E-2-Tetradecen-1-ol / 10-Methyl-E-11-tridecen-1-ol propionate	0.703
19	20.095	Dodecanoic acid, 3-hydroxy-	0.143
20	20.215	9-Hexadecenoic acid / 12-Hydroxydodecanoic acid	0.130
21	21.681	Oxirane, [(dodecyloxy)methyl]-	0.107
22	22.006	Tetradecanal / Oxirane, tetradecyl- / Dodecana/ Pentadecanal-	0.264
23	23.172	Tetradecanoic acid / Myristic acid / Octadecanoic acid	0.094
24	23.397	Dodecanoic acid, 3-hydroxy-	0.049
25	23.472	trans-13-Octadecenoic acid	0.052
26	23.747	Undecanoic acid / Dodecanoic acid / Undecanal / Dodecanal	0.111
27	24.042	3,7,11,15-Tetramethyl-2-hexadecen-1-ol / 9-Eicosyne/ Oxirane, hexadecyl-	1.031
28	24.147	2-Pentadecanone, 6,10,14-trimethyl- / 12-Octadecenal	0.070
29	24.387	Octadecanal / Oxirane, dodecyl-	0.077
30	24.637	Oxirane, tetradecyl-	0.137
31	24.837	Linoleic acid	0.047
32	24.917	Cyclopentadecanone, 4-methyl- / 2-Hexadecanol / 1-Hexadecanol, 2-methyl-	0.072
33	25.152	E-2-Tetradecen-1-ol	0.088
34	25.878	n-Hexadecanoic acid / Palmitic acid	1.167
35	26.053	Hexadecanoic acid, ethyl ester/ Octadecanoic acid, ethyl ester/ Tridecanoic acid	0.200
36	27.353	Phytol	0.764
37	27.778	9,12,15-Octadecatrienoic acid, (Z,Z,Z)- / 8,11,14-Eicosatrienoic acid, (ZZZ)-	0.817
38	27.883	9,12,15-Octadecatrienoic acid, ethyl ester, (Z,Z,Z)-	0.642
39	27.993	Octadecanoic acid	0.427
40	28.144	Cyclopropanetetradecanoic acid, 2-octyl-, methyl ester / Eicosanoic acid	0.239
41	28.334	17-Octadecynoic acid / Ethanol, 2-(9-octadecenyloxy)-, (Z)-	0.096
42	28.704	Z-(13,14-Epoxy)tetradec-11-en-1-ol acetate	0.046
43	29.119	Erucic acid / 7-Hexadecenal, (Z)-	0.063
44	29.399	cis-10-Nonadecenoic acid / cis-11-Eicosenoic acid	0.054
45	29.539	cis-13-Octadecenoic acid	0.046
46	30.810	Oleic Acid	0.054
47	31.045	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester, 2,3-dihydroxypropyl ester	0.247
48	31.110	10-Undecenoic acid, octyl ester / Octadecanal, 2-bromo-	0.168
49	32.400	1-Eicosanol / Hexacosanol, acetate / 1-Docosene / 1-Octacosanol	0.637
50	32.690	cis-10-Heptadecenoic acid / n-Tetracosanol-1	0.246

Table 2: Effect of ethanolic extract of *M. parviflora* (MP) leaves on growth performance and survival rate (Mean ± SE) n=20.

Items	G1	G2	G3	G4	G5
Initial weight (g)	100.40 ± 1.55 a	100.60 ± 1.60 a	100.40 ± 1.50 a	100.80 ± 1.23 a	100.40 ± 1.70 a
After 30 days (g)	122.70 ± 1.62 a	120.40 ± 1.34 a	139.30 ± 1.15 a	143.40 ± 1.74 a	128.80 ± 1.32 a
Final weight after 60 days	156.67 ± 0.88 a	155.93 ± 1.46 a	182.53 ± 1.81 c	196.10 ± 1.42 d	161.90 ± 1.59 b
Weight gain (g)	56.27 ± 0.88 a	55.33 ± 1.46 a	82.13 ± 1.81 c	95.30 ± 1.42 d	61.50 ± 1.59 b
Feed intake (FI) g/fish	270.2 ± 12.57 a	269.7 ± 10.70 a	285.7 ± 10.67 c	293.9 ± 9.94 d	271.5 ± 11.07 b
Feed conversion ratio (FCR)	4.80 ± 1.09 d	4.85 ± 1.00 d	3.47 ± 0.94 b	3.07 ± 0.88 a	4.41 ± 1.17 c
Specific growth rates (SGRs)	0.75 ± 0.03 a	0.74 ± 0.03 a	1.01 ± 0.04 c	1.13 ± 0.02 d	0.81 ± 0.03 b
Weight gain %	56.04 ± 1.18 a	55.30 ± 1.20 a	81.08 ± 1.50 c	95.31 ± 1.12 d	61.25 ± 1.34 b
Survival %	100	40	80	80	70

The various letters in the same row indicate statistically significant differences when (P<0.05).
G1= negative control G2= positive control G3= 0.25 g MP G4= 0.5 g MP G5= 1 g MP.

Table3: Effect of ethanolic extract of *M. parviflora* (MP) leaves on nitric oxide (NO) concentration and lysozyme enzyme activity (Mean ± SE) n=5.

G	Periods (days)	Macrophage NO conc. micromolar (µMol)	Lysozyme activity (µMol)
G1	After 30 days	27.93 ± 1.45 a	108.53 ± 1.33 a
	After 60 days	30.93 ± 0.77 a	117.30 ± 1.01 a
	Post challenge	32.89 ± 0.98 a	127.43 ± 1.17 a
G2	After 30 days	30.74 ± 1.13 a	110.15 ± 0.84 a
	After 60 days	31.74 ± 0.96 a	118.50 ± 1.70 a
	Post challenge	40.15 ± 1.33 b	135.72 ± 1.72 b
G3	After 30 days	39.62 ± 0.82 c	112.33 ± 0.64 ab
	After 60 days	38.26 ± 0.80 c	122.78 ± 0.92 ab
	Post challenge	67.34 ± 0.99 d	137.67 ± 0.85 bc
G4	After 30 days	34.79 ± 1.45 b	128.45 ± 2.23 c
	After 60 days	35.33 ± 0.98 b	139.33 ± 2.62 c
	Post challenge	69.44 ± 0.88 d	154.22 ± 2.66 d
G5	After 30 days	37.15 ± 1.18 bc	117.15 ± 3.13 b
	After 60 days	29.44 ± 1.04 a	128.32 ± 3.79 b
	Post challenge	54.79 ± 1.92 c	143.23 ± 3.72 c

The various letters in the same colon of the same period indicate statistically significant differences when (P<0.05).
G1= negative control G2= positive control G3= 0.25 g MP G4= 0.5 g MP G5= 1 g MP.

Table 4: Effect of ethanolic extract of *M. parviflora* (MP) leaves on total leucocytic count (T.L.C) x10^3/ul and differential leucocytic count (D.L.C) (Mean ± SE) n=5.

G	Periods	TLC x103 /ul	N %	E%	B%	L%	M%
G1	After 30 days	24.33 ± 1.38 a	21.00±2.08 b	10.40 ±0.20 c	3.40 ±0.40 b	57.80 ±2.00 a	7.40 ±0.20 a
	After 60 days	25.17 ± 1.14 a	21.60±1.40 b	9.00 ±0.00 c	4.20 ± 0.20 c	58.00 ±1.00 a	7.20 ±0.40 a
	Post challenge	24.96 ± 1.21 a	21.60±1.20 b	9.40 ±0.40 c	4.60 ±0.40 c	57.00 ± 2.00 a	7.40 ±0.20 a
G2	After 30 days	25.14 ± 1.22 a	20.80±2.08 b	10.20 ±0.40 b	3.60 ±0.40 b	57.80 ± 2.00 a	7.60 ±0.20 a
	After 60 days	25.46 ± 1.45 a	20.80±1.20ab	8.40 ± 0.20bc	3.60 ±0.40 bc	60.60 ± 2.00 a	6.60 ±0.20 a
	Post challenge	41.11 ± 1.58 d	18.60±2.91 a	5.20 ±0.20 a	0.20 ±0.60 a	67.40 ± 3.00 d	8.60 ±0.20 b
G3	After 30 days	28.67 ± 1.18 b	21.20±1.80 b	8.00±0.00 a	2.20 ±0.20 a	60.00 ± 2.00 b	8.60 ±0.20 b
	After 60 days	29.62 ± 1.32 b	20.00±1.20 a	7.60 ±0.33 b	1.60 ±0.40 a	62.60 ± 1.00 b	8.20 ±0.20 b
	Post challenge	31.79 ± 1.12 b	18.60±2.91 a	6.40 ±0.88 ab	2.20 ±0.67b	64.20 ± 2.00 b	8.60 ±0.20 b
G4	After 30 days	29.14 ± 1.31 b	19.20±1.80 a	8.00 ±0.40 a	2.20 ±0.20 a	62.00 ± 2.00 c	8.60 ±0.20 b
	After 60 days	31.23 ± 1.45 b	21.00±1.40 b	6.40 ±0.20 a	0.60 ±0.20 a	64.00 ± 2.00 b	8.00 ±0.20 b
	Post challenge	37.18 ± 1.24 c	18.60±2.91 a	6.20 ±0.88 a	0.20 ±0.60 a	66.40 ± 2.00 c	8.60 ±0.20 b
G5	After 30 days	29.85 ± 1.25 b	20.40±1.60 b	8.00 ±0.40 a	2.60 ±0.20 a	62.00 ± 1.00 c	7.00 ±0.20 a
	After 60 days	32.59 ± 1.21 c	20.00±1.60 a	7.00 ±0.20 a	2.20 ±0.20 b	63.00 ± 2.08 b	7.80 ±0.40 b
	Post challenge	35.94 ± 1.37 c	20.60±1.20 b	5.60 ±0.20 a	0.60 ±0.20 a	64.80 ± 1.00 b	8.40 ±0.20 b

The various letters in the same colon of the same period indicate statistically significant differences when (P<0.05).
G1= negative control G2= positive control G3= 0.25 g MP G4= 0.5 g MP G5= 1 g MP.
Neutrophil (N), eosinophil (E), basophil (B) lymphocyte (L) and monocyte (M), (% of T.L.C).

Table (5): Effect of ethanolic extract of *M. parviflora* (MP) leaves on some of serum biochemical parameters (Mean ± SE) n=5.

G	Periods	Total protein g/dl	Albumin (g/dl)	Globulin (g/dl)	A/G ratio	ALT (u/l)	AST (u/l)	ALP (u/l)
G1	After 30 days	3.76 ± 0.09 b	1.59 ± 0.02 b	2.16 ± 0.07 a	0.74 ± 0.01 ab	43.88 ± 0.58 a	33.11 ± 0.73 ab	17.28 ± 0.37 a
	After 60 days	4.11 ± 0.03 b	1.52 ± 0.02 b	2.59 ± 0.01 b	0.58 ± 0.01 a	51.85 ± 1.00 a	39.07 ± 0.59 ab	23.45 ± 0.53 b
	Post challenge	4.24 ± 0.02 b	1.59 ± 0.01 b	2.65 ± 0.04 b	0.60 ± 0.01 a	47.61 ± 0.40 a	84.07 ± 0.59 a	12.99 ± 0.53 a
G2	After 30 days	3.69 ± 0.11 b	1.61 ± 0.01 b	2.08 ± 0.12 a	0.78 ± 0.04 b	44.20 ± 0.41 a	33.73 ± 0.46 ab	16.60 ± 0.35 a
	After 60 days	4.18 ± 0.03b	1.50 ± 0.02 b	2.68 ± 0.05 b	0.56 ± 0.01 a	52.11 ± 0.67 a	40.06 ± 0.78 b	24.49 ± 0.75 b
	Post challenge	3.18 ± 0.03 b	1.30 ± 0.02 a	1.88 ± 0.05 a	0.69 ± 0.02 a	63.91 ± 0.72 d	102.06 ± 0.78 c	22.25 ± 0.75 c
G3	After 30 days	3.59 ± 0.12 b	1.63 ± 0.02 b	1.96 ± 0.13 a	0.84 ± 0.06 b	43.50 ± 0.26 a	31.94 ± 0.64 a	17.75 ± 0.46 a
	After 60 days	4.06 ± 0.10 b	1.53 ± 0.02 b	2.53 ± 0.08 b	0.60 ± 0.01 a	50.23 ± 0.28 a	37.32 ± 0.68 a	19.10 ± 0.78 a
	Post challenge	4.06 ± 0.10 b	1.58 ± 0.02 b	2.49 ± 0.09 b	0.64 ± 0.02 a	46.20 ± 0. 48 a	81.91 ± 0.68 a	12.31 ± 1.24 a
G4	After 30 days	3.80 ± 0.08 b	1.66 ± 0.05 b	2.14 ± 0.05 a	0.78 ± 0.03 b	46.74 ± 0.13 b	34.25 ± 0.42 b	17.41 ± 0.35 a
	After 60 days	3.94 ± 0.05 b	1.49 ± 0.02 b	2.46 ± 0.04 b	0.61 ± 0.00 a	54.29 ± 0.37 b	80.37 ± 1.22 c	27.09 ± 0.53 c
	Post challenge	4.14 ± 0.18 b	1.55 ± 0.01 b	2.59 ± 0.18 b	0.60 ± 0.04 a	50.29 ± 0.47 b	93.54 ± 1.12 b	16.63 ± 0.53 b
G5	After 30 days	3.24 ± 0.11 a	1.25 ± 0.02 a	1.99 ± 0.09 a	0.63 ± 0.02 a	63.45 ± 0.26 c	71.36 ± 0.75 c	22.38 ± 0.48 b
	After 60 days	3.23 ± 0.13 a	1.38 ± 0.01 a	1.85 ± 0.13 a	0.76 ± 0.05 b	57.86 ± 0.32 c	96.95 ± 0.59 d	29.44 ± 0.79 d
	Post challenge	3.06 ± 0.07 a	1.24 ± 0.03 a	1.82 ± 0.09 a	0.68 ± 0.05 a	54.43 ± 0.46 b	92.94 ± 0.59 b	18.98 ± 0.78 b

The various letters in the same colon of the same period indicate statistically significant differences when (P<0.05).
G1= negative control G2= positive control G3= 0.25 g MP G4= 0.5 g MP G5= 1 g MP.

Table 6: Effect of ethanolic extract of *M. parviflora* (MP) leaves on antioxidant enzymes, serum urea and creatinine (Mean ± SE) n=5.

G	Periods	MDA (nmol/ml)	SOD (u/ml)	GSH (mg/dl)	Urea (mg/dl)	Creatinine (mg/dl)
G1	After 30 days	14.69 ± 0.18 b	178.14 ± 1.17 a	129.59 ± 1.27 c	10.41 ± 0.17 b	0.33 ± 0.02 b
	After 60 days	15.36 ± 0.20 b	185.54 ± 0.97 c	114.75 ± 1.18 d	10.92 ± 0.17 b	0.48 ± 0.02 b
	Post challenge	11.88 ± 0.20 b	178.14 ± 1.26 b	115.02 ± 1.27 b	12.70 ± 0.17 b	0.75 ± 0.04 b
G2	After 30 days	15.01 ± 0.19 b	178.41 ± 1.61 a	129.81 ± 1.67 c	10.39 ± 0.20 b	0.34 ± 0.01 b
	After 60 days	15.34 ± 0.21 b	185.65 ± 1.75c	114.80 ± 1.74 d	10.94 ± 0.19 b	0.49 ± 0.02 b
	Post challenge	16.89 ± 0.19 d	155.08 ± 1.99 a	109.01 ± 1.67 a	14.77 ± 0.18 d	1.09 ± 0.01 d
G3	After 30 days	11.94 ± 0.18 ac	309.53 ± 1.58 b	140.94 ± 1.25 d	4.26 ± 0.11 a	0.26 ± 0.00 a
	After 60 days	10.62 ± 0.15 a	106.88 ± 1.42 a	68.85 ± 1.26 b	10.36 ± 0.08 a	0.36 ± 0.01 a
	Post challenge	10.34 ± 0.18 a	216.53 ± 3.47 c	126.37 ± 1.25 d	12.14 ± 0.08 a	0.63 ± 0.01 a
G4	After 30 days	15.91 ± 0.14 d	328.38 ± 2.04 d	96.30 ± 0.96 a	10.12 ± 0.16 b	0.29 ± 0.01 a
	After 60 days	18.90 ± 0.26 c	135.10± 1.48 b	40.65 ± 1.12 a	11.09 ± 0.15 b	0.68 ± 0.01 c
	Post challenge	15.42 ± 0.25 c	228.04 ± 2.33 d	120.43 ± 0.96 c	12.87 ± 0.35 b	0.95 ± 0.01 c
G5	After 30 days	15.47 ± 0.21 cd	318.74 ± 1.33 c	100.64 ± 1.00 b	11.03 ± 0.10 c	0.34 ± 0.01 b
	After 60 days	18.71 ± 0.09 c	182.21 ± 1.79 c	104.48 ± 1.04 c	12.39 ± 0.09 c	0.95 ± 0.01 d
	Post challenge	15.23 ± 0.29 c	219.56 ± 0.91 c	124.77 ± 1.00 d	14.18 ± 0.28 c	0.94 ± 0.01 c

The various letters in the same colon of the same period indicate statistically significant differences when ($P<0.05$). G1= negative control G2= positive control G3= 0.25 g MP G4= 0.5 g MP G5= 1 g MP.

DISCUSSION

Treatment of bacterial diseases in fish aquaculture by antibiotic medicated ration is a usual practice but, this is usually costive and may be ineffective due to multiple and incorrect use of antibiotics develops a resistance to most of these compounds. Therefore, the search for alternative methods of treatment has become a necessity that cannot be neglected. Plant extracts consider a safe, natural, inexpensive and effective method of treatment. So, this study was focused on the role of *M. parviflora* (little mallow) extract in improvement the performance and immunological response of Nile tilapia and so combating and controlling the infection in addition to its antibacterial effect against *P. fluorescens*.

This study investigated the various constituents and the pharmacological activities of ethanolic extract of *M. parviflora* leaves using GC-MS technique, searching for its active constituents responsible for their therapeutic efficacy; antimicrobial, immunological and antioxidant properties. The major compounds (Fig. 1 and Table 1) of *M. parviflora* were; Hexadecanoic acid, 1-(hydroxymethyl)-1,2-ethanediyl ester and Ascorbic acid 2,6-dihexadecanoate (45.027%) followed by Oleic Acid in addition to Octadecanoic acid, octadecyl ester / tetradecenoic acid (26.338%), then Triarachine and Hexadecanoic acid, 2-(octadecyloxy) ethyl ester (10.487%). Other vital fatty acids (FAs), esters and compounds constituted an important portion of the extract were also detected; Thiocyanic acid, ethyl ester, Vaccenic acid, Erucic acid, Tetradecanoic acid, Octadecanoic acid, Linoleic acid, Myristic acid, Palmitic acid, Eicosanoic acid and Phytol. Our findings coincide to some extent with Al- Qarawi and Al-Obaidi (2018) who found that methanolic extract of *M. parviflora* leaves contained major active compounds such as 21,9-Octadecadioenaic acid (z,z), methyl-ester and 10,13 Octadecadioenaic acid,-methyl-ester-(70.58%), Hexadecanoic acid, methyl ester (8.42%), 1,2-Benzisothiazole, 3-(hexahydro-1H-azepin-1-y1) -,1,1-dioxide (3.32%), Hexasiloxane, 1,1,3,3,5,5,7,7 and 9,9,11,11 dodecamethyl-(2.29-%), Methyl 10-trans, 12-cis-octadecadienoate (1.59%) and Trans-13-octadecenoic acid, methyl

ester (1.55%) besides others in low doses. Moreover, Abdel-Ghani *et al.* (2013) analyzed light petroleum fraction of *M. parviflora* using GC-MS and found β-amyrin, mixture of β-sitosterol and stigmasterol, ergosterol and β-sitosterol- O-glucoside, α-amyrine, cholesterol and campasterol. Methylated fatty acids (FAs) indicated the presence of (14-FAs), while the major were linolenic, palmitic and linoleic acids and accounting 28.25; 26.5 and 24.73%, respectively. Chloroform fraction gave ethyl vanillin, chlorophyll A and B. The ethyl acetate fraction revealed kampeferol-3-(6''-p-coumaroyl-O-β-D-glucoside (tribuloside). Authors attributed the anti-inflammatory properties of this extract to its bioactive compounds. Regarding to *M. sylvestris*, Tabaraki *et al.* (2012) found that, the main compound in its methanolic extract was 2-methoxy-4-vinylphenol. Also, Razavi *et al.* (2011) found that *M. sylvestris* anthocyanins reduced plasma total triglycerides (TTG), cholesterol in addition to protected animals from ethanol provoked gastritis due to its high mucilage amount. As well, El Khoury *et al.* (2020) identified major compounds in methanolic extract of *Malva pseudolavatera* as (Z,Z,Z)-9,12,15-Octadecatrienoic acid-methyl-ester plus another Omega-3 fatty acid ester; Phytol, Hexadecatrienoic acid, and Phytosterols, particularly γ-sitosterol and stigmasterol that showed signs of selective-toxicity on various types of cancer cells with no effect on normal cells besides their anti-inflammatory and antioxidant effects.

Despite the traditional use of *M. parviflora* in medicine, a small number of pharmacological studies are reviewed evaluating its therapeutic possessions as antibacterial activity against fish infections. This experiment is conducted on the antibacterial activity of the ethanolic extract of *M. parviflora* leaves on *P. fluorescens* in fish. About commercial antibiotics, Algammal *et al.* (2020) found that 55.5% of *P. aeruginosa* isolates from *O. niloticus* farms in Egypt, showed evidence of multi-drug resistance (MDR) to cefotaxime, amoxicillin, tetracycline and gentamicin in direct relation to the antibiotic resistance and encoded virulence genes provided a forewarning to the misuse of antimicrobials. In our antibiogram, *M. parviflora* extract showed increased IZD on *P. fluorescens* ranged from 11 ± 0.33 to 16 ± 0.40 mm. Minimum inhibitory and minimum bactericidal concentrations (MIC and MBC) values were 3.90 and 62.50 mg/ml respectively. Our results indicated the susceptibility of *P. fluorescens* to the used extract when compared with IZDs of Gentamicin (17 ± 0.20) and Ciprofloxacin (22 ± 0.36) mm. The antibacterial activity of both leaves and flowers of Malva sp. including *M. parviflora* against saprophytic and pathogenic bacteria otherwise gram positive or negative were stated by Shale *et al.* (2005); Mihaylova *et al.* (2014). Also, Zare *et al.* (2012) found that aerial parts ethanolic extract of *Malva sylvestris* and *Malva neglecta* had the highest antibacterial activity and the best MIC values compared to other solvents; chloroform or water against *S. aureus, P. vulgaris, P. aeruginosa,* and *S. pyogenes*. As well, Rasheed *et al.* (2017) reported similar results for *M. parviflora*. Also, Klūga *et al.* (2017) found strong antimicrobial effect of ethanolic extract of *Malva mauritiana* leaves against *Pseudomonas oryzihabitans* and *Pseudomonas alcaligenes* than other bacteria with IZD (6.67±1.53 and 4.67±0.58 mm) respectively and assured the abundance of *P. fluorescens* as opportunistic pathogens of fish microflora. Minimum inhibitory concentration (MIC) was also used as a comparative index for antimicrobial agents. Shadid *et al.* (2021) reported that hexane, methanolic, aqueous and acetone *M. parviflora* extracts revealed antibacterial activity against P. aeruginosa and MICs were 6.25, 6.25, 3.125 and 12.5 mg/ml respectively. Also, Afifi (2016) recorded the antibacterial effect of ethanolic *M. sylvestris* leaves extract, at 150µg/disc against *Aeromonas veronii, A. hydrophilla A. jandaei*, and *A. caviae* except *A. sobria*. The maximum IZDs were 55 and 54 mm for *A.*

caviae and *A. hydrophila* recpectively and MIC was 50 µg/ml. Razavi *et al.* (2011) mentioned that methanol extract of *M. sylvestris* leaves displayed high bactericidal activity against; *E. faecalis*, *E. coli*, *S. agalactiae* and *S. aureus* with a comparable efficacy to erythromycin, gentamycin and amphotericin, its MIC values ranged from 192-256 µg/ml. Consequently, the author concluded that it can be applied as a chemotherapeutic or a chemopreventive means. Dugani *et al.* (2016) attributed the pharmacological activity of *M. parviflora* leaves extract to the existence of a variety of phytoconstituents as steroids, anthraquinones, phenolic compounds, flavonoids, tannins, glycosides and particularly the coumarins of the leaves which present in alcoholic extract than aqueous extract with a dose dependent protective effect, in addition to its inhibitory effect on prostaglandins synthesis. As known, when infection starts the inflammatory response released several mediators besides stimulation of the neutrophils and macrophages (Martins *et al.*, 2017). Furthermore, Ododo *et al.* (2016) stated that the ethanolic besides chloroform extracts of *M. parviflora* root bark own active compound to suppress both *S. aureus* and *E. coli* with IZDs (18 ± 3.20 and 15 ± 0.41 mm) along with MIC (15 and 20 mg/ml), respectively. Bouriche *et al.* (2011) stated that *M. parviflora* leaves had as well less lipophilic constituents which are dispersed in both methanol and aqueous extracts in charge of its effects which might be associated with the existence of water-soluble phytochemicals; flavones, organic acids and quinones. The discrepancy in the IZD or MIC values obtained in the present study and other previous reports may be attributed to the Pseudomonas sp., Malva sp. or several variables which manipulate the bioactive plant ingredients as solvent type.

Our results proved that addition of little mallow extract in *O. niloticus* fish ration has a positive growth promotion effect on all treated groups than the control especially G4 (Table 2). FCR values of G3 and G4 decreased significantly than the control. Growth promotion may also supply an early protective immune response against infections. This improvement of growth performance and immune response due to the extract, raise the resistance and survival rates of all administered groups challenged with *P. fluorescens* infection besides its direct antibacterial activity. Similarly, Bilen *et al.* (2019) reported that *M. sylvestris* aqueous methanolic extract promoted the growth and immunity in gilthead sea bream plus European sea bass and elevate survival rate against the infection with *V. anguillarum*. Also, Rashidian *et al.* (2019) found that common mallow flowers extract can elevate the performance and immunity of rainbow trout.

For sustaining disease resistance in cultured fish, immunostimulants have been used as dietary supplements (Vallejos-Vidal *et al.*, 2016) and their effect has been concentrated mainly on the assessment of non-specific immune stricture and as a result, on the consequences of these treatments on the innate immunity. In this study, after 30, 60 days and post challenge (Table 3) no significant difference was recorded in lysozyme activity and NO level between G3 and G5, while the highest value recorded in G4. The phagocytic and lysozyme actions are good markers for fish immunological status especially during periods of infection. Positive control post challenge showed a significant increase in both items than negative control. This probably attributed to the high leukocyte production in infected group regarding to the fact that fish lysozyme is mainly produced by neutrophils and macrophages (Bussolaro *et al.*, 2008). All treated groups showed a significant increased than both controls before and after challenge point to an immune modulation. Our results agreed with many studies that different composites in herb extracts can straightforwardly trigger the increase of non-specific immune parameters and support to fish immune system. Ramírez-Serrano *et al.* (2019) reported the ability of hydroalcoholic *M. parviflora* extract to activate

macrophages through its fractions like daucosterol. Also, Afifi (2016) stated that ethanolic *M. sylvestris* leaves extract can act as immune stimulant in *O. niloticus* enhanced lysozome and respiratory burst mechanism when used at 0.5 and 1% of ration for 4 weeks than control, while no significance difference between both treated groups, besides increased the relative levels of protection after challenge with pathogenic *A. sobria* to be zero mortality compared with 50% in positive group. Moreover, several studies as Bilen *et al.* (2019) on sea bream and sea bass and Bilen *et al.* (2020) on common carp concluded that methanolic extract of *M. sylvestris* at (0.5, 1 g/kg ration) for 45 and 60 days, respectively was suitable applicant herb to support lysozyme, phagocytic and myeloperoxidase activities as an important indicators to non-specific immune defense response against pathogens so; *M. sylvestris* exhibited a positive role in immunological responses as an inexpensive natural immunomodulators to render resistance to diseases providing a protection against *V. anguillarum* challenge. Our results could be attributed to various flavonoids components contained in Malva (Saad *et al.*, 2017). Flavonoids have direct effect to lysozyme level (Yang *et al.*, 2012). As well, the positive immune effects may be attributed to antioxidant of Malva such as polyphenols, vitamins E, C, B-carotene and other vital phytochemicals based on their capability to scavenge diverse free radical resulted in protection of biological molecules in opposition to oxidation (Rackova *et al.*, 2009).

Hematology and clinical chemistry analysis can provide substantial diagnostic information however they not used regularly in fish medicine (Hrubec *et al.*, 2000), D.L.C belong to important distinctiveness of fish health state and helpful in immune system evaluation. Our results revealed that all treated groups after 30, 60 days and post challenge (Table 4) had a significant increase in T.L.C, lymphocyte and monocyte counts than negative control while in positive control increased post challenge only. As reported by Zexia *et al.* (2007), the 3 types of granulocytes described in higher vertebrates are also present in fish. By contrast with mammals, fish belong to the lymphocytic group and neutrophils may represent less than 5% of leukocytes and the major part of them is stored in kidney (Havixbeck *et al.*, 2016). Regarding to positive control, similar results of leucocytosis were recorded after bacterial infection in Tilapia in order to tackle the infection (Mazrouh *et al.*, 2015) which, may be attributed to lymphocytosis and neutrophilia. As well Afiyanti *et al.* (2018) estimated the leukocytes and D.L.C in carp 3 days after *A. salmonicida* infection and found a significant leukocytosis, eosinophilia, neutrophilia, lymphocytosis and monocytosis and non-significant basophilic count. In treated groups the extract was capable of enhancing the act of the immune system by generating more leucocytes, as a result giving the fish more ability to restrain the bacterial growth in the fish body. Similarly, lymphocyte increased in *O. mykiss* exposed to *V. anguillarum* or its extra cellular content (Lamas *et al.*, 1994). Conversely, Bilen *et al.* (2020) recorded no changes in hematological parameters of all common carp groups treated with aqueous methanolic extract of *M. sylvestris* (0.1, 0.5, and 1g/kg) for 45 days. Meanwhile the effect of the extract on hematological profile may depend upon extract type, fish sp., age and other circumstances. Fish have several kinds of phagocytic leukocytes in the peritoneal cavity, and different tissues. Plant extracts are full of vital phytochemicals may enhance monocytes plus granulocytes; macrophages and neutrophils, improve action of non-specific immune responses. Furthermore, activated neutrophils and macrophages in the fish blood as well amplify the number of reactive oxygen and nitrogen intermediates (ROIs and RNIs), which act as toxic to bacteria (Hardi *et al.*, 2019).

By estimation of liver enzyme markers as AST, ALT and ALP (Table 5), our results after 30 and 60 days revealed that G4 and G5 showed a significant increased than G1,

while there is no significant difference between G3 and G1 indicating that considerable increased in enzyme activities is a dose dependent. Post challenge, positive control showed a significant increased than negative control. Release of liver enzymes indicates mitochondrial damage and represents the loss of functional uprightness due to the cellular enzymes' leakage from the cell membrane of the hepatocyte. Our results evidenced that negative impacts of *M. parviflora* extract on liver appeared to be a concentration dependent and this coincide with Bilen *et al.* (2020) who recommended that more pharmacological and toxicological studies are needed on common mallow for drug safety dose for fish health. Also, Algammal *et al.* (2020) recorded that liver was the most prominent bacteriologically infected and affected organ with *P. aeruginosa* pursued by kidney then spleen in naturally infected fish where it caused grave illness together with hemorrhagic septicemia, abdominal dropsy, friable pale liver along with congested kidney. Regarding to the extract dose effect, Hussain *et al.* (2014) reported that hepatoprotective effect of *M. sylvestris* at (300 plus 600 mg/kg BW), which used to treat induced hepatic intoxication found in a dose dependent manner, restoring normal function ability of liver specially at 600 mg/kg with no significant difference recorded compared to silymarin (100 mg/kg) treated group. The authors attributed this effect to the active phytocomponents that might reinstate the glutathione values in hepatocytes and its functional integrity. Glutathione is considered one of the key antioxidants protect the liver from toxic actions. The discrepancy in the hepatoprotective effect of various ascending doses of the used *M. parviflora* ethanolic extract and other studies may be referred to the affected species, duration of treatment, type of the Malva sp. or the used extract solvents. Our results about TP, albumin, globulin and A/G ratio after 30 and 60 days provoked no significant difference among G3 and G4 when compared to G1 in most of these parameters while G5 showed a significant decrease. Post challenge G2 and G5 showed a significant decrease than G1. Results of positive control are agreed with Adonova *et al.* (2014) who recorded hypoalbuminemia post *P. aeuroginosa* infection. The authors endorsed the reduction of albumin value to enhanced vascular permeability caused by bacterial toxins resulting in albumin passage to the adjacent interstitial tissue. In this respect also, Saad *et al.* (2014) discussed the decrease in protein and globulin that can explain the drastic effect of Pseudomonas infection on immunity of infected fish with afterward increased the damaging effects of bacterial disorders. Our results come partially in agreement with Hajyani and Modaresi, (2016) who reported that ethanolic extract of *M. sylvestris* in 50, 100, 200 mg/kg for 20 days revealed a significant decreased in blood albumin and increased in the gamma interferon for all doses while, the amount of β-globulin increased only in 50 and 100 mg/kg treated groups than the control referring to its ability to stimulate the immune cellular response.

Antioxidant reactions of living organisms are important defense means for defending against stressors or infections (Yin *et al.*, 2014). Pretreatment with all *M. parviflora* extract doses resulted in dose dependent effects on the oxidative stress biomarkers. Various effects were recorded (Table 6) in MDA levels after 30 and 60 days; the best decrease was in G3 than negative control, while G4 and G5 revealed no significant difference between each other. All treated groups elicited a significant decrease than G2. SOD, after 30 days all treated groups showed a significant increase than G1 then after 60 days, only G5 showed no significant difference than G1. Reduced glutathione (GSH) after 30 days, only G3 showed a significant increase than G1 then after 60 days; the remarkable increase was noticed in G5 while all treated groups were significantly less than G1. Post challenge, positive control increased significantly MDA and decrease in both SOD and GSH levels than negative control with no

significant difference between G3 along with G5, while all treated groups were increased significantly than both controls. Our data propose that ethanolic extract of *M. parviflora* contained good radical scavenger compounds. As well our GC-MS investigation confirmed the presence of ascorbic acid in the highest area percent as main constituent of *M. parviflora.* Moreover, pharmacological studies exist for *M. parviflora* extracts displayed its antioxidant activity and anti-inflammatory properties attributed these to oleanolic acid, scopoletin, and tiliroside fractions (Lagunas-Herrera *et al.*, 2019), sterols, flavonoids, and fatty acids (Bouriche *et al.*, 2011) or to polyphenols and proanthocyanidins (Afolayan *et al.*, 2010) which act via scavenging of free metal ions, thus, depressing transition of metal ion depending oxidative processes, in addition to its usage in treatment of liver injuries and inflammation. Also, Paul (2016) reviewed that *M. sylvestris* contains tannins, vitamins (A, C, and E), folic acid, niacin, polyphenols (anthocyanins), scopoletin, malvone A (naphthoquinone) malvaline, malvidin, malvin, flavonoids, polysaccharides (mucilage) and coumarins. As well our findings come in agreement with Perez Gutierrez, (2012) who reported that *M. parviflora* leaves extract can well hinder lipid abnormalities and oxidants' stress. Also, Adam *et al.* (2018) found that *M. parviflora* leaves have the highest antioxidant actions among other plants without any cytotoxicity. It may serve as a resource for further expansion of natural antioxidant agents due to its total phenol and flavonoid amounts. As well Shadid *et al.* (2021) stated the presence of safer and inexpensive antioxidants in hexane, methanolic, aqueous, and acetone *M. parviflora* extracts due to their phenolic contents and radical scavenging activity. In another study, Farhan *et al.* (2012) reported that scavenging activity was 88% for *M. parviflora* leaves and comparable to the ascorbic acid as antioxidant. To boot, Bouriche *et al.* (2011) found that antioxidant activities of the methanol extract of *M. parviflora* leaves were superior to aqueous extract; it exerted a strong scavenging activity and chelated ferrous ions in a dose dependent manner. Thus, *M. parviflora* leave have great prospective as an interesting source for natural health agents.

Serum urea and creatinine consider as biomarkers for kidney functions in fish (Dos Santos *et al.*, 2017). Our results after 30 and 60 days (Table 6) revealed that G4 and G5 increased significantly than G1 while, no significant difference between G3 and G1 indicating that considerable increase in levels of these parameters is a dose dependent manner. Post challenge, positive control showed a significant increase than negative control. Dos Santos *et al.* (2017) reported similar findings about *P. aeruginosa* infection. Furthermore, Eissa *et al.* (2010); Devakumar *et al.* (2013); Magdy *et al.* (2014); Derwa *et al.* (2017) recorded marked histopathological alterations in hepatic and renal tissues after *P. aeruginosa* infection. Aekanurmaningdyah and Kurniasih, (2018) attributed these cellular degenerative changes to manipulate of bacterial toxins, enzymes which had hemolytic and proteolytic activities, and bioactive extracellular agents promoting tissue degeneration, liver necrosis, hemorrhage, and renal nephrosis. Regarding to the extract dose effect, Babaei Zarch *et al.* (2017) reported a nephroprotective effect of *M. sylvestris* on renal damage of induced nephrotoxicity and attributed this to the antioxidant activity of the bioactive compounds involved in the plant and its efficacy was dose dependently. All doses of 100, 200 and 400 mg/kg BW /day for 1 week showed an improvement in estimated parameters and it was significant only at 400 mg/kg/day. The authors found that *M. sylvestris* extract pretreatment significantly decreased BUN, creatinine and MDA levels besides increased catalase and GSH activities. These effects were confirmed histopathologically and were comparable with those of vitamin C (10 mg/kg) as a strong antioxidant, which applied by the authors as a control. Similar positive histopathological effects of *M. parviflora*

aqueous extract reported by Farhan and Mohammed, (2020) on both liver and kidney tissues at 300 and 500 mg/kg BW/30 days against potassium permanganate as oxidizing substance. Furthermore, Marouane *et al.* (2011); Nabavi *et al.* (2012); Zhang *et al.* (2014) confirmed the protective effect of flavonoids rich substance, phenolic compound and curcumin on kidney functions through their antioxidant effects. The discrepancy in the nephroprotective effect of various doses of the used *M. parviflora* ethanolic extract and other studies may be related to the affected species, duration of treatment, type of the Malva sp. or the used extract solvents.

CONCLUSION

It could be fulfilled that *M. parviflora* leaves ethanolic extract is a promising therapeutic agent it can be used as antibacterial with effective an immunmodulator activity against *P. fluorescens* in Nile tilapia fish. It exerts significant improvement on growth performances, increases survival rate and non-specific immune activities, suppresses oxidative stress and lipid peroxidation. It has a pharmacological prospective as a valuable natural product that can be utilized as an effective therapeutic supplementation of Tilapia fish ration. Its effects appeared in a dose dependent manner. The dose of 0.25 g/kg ration recorded no side effects on liver or kidney functions, while 0.5 g/kg gave the highest weight gain. As well, both doses of 0.25 and 0.5 g/kg were effective enough and increased immunity and survival rate to 80%.

REFERENCES

Abdel-Ghani, A.E.; Hassan, H.M. and El-Shazly, A.M. (2013): Phytochemical and biological study of Malva parviflora L. grown in Egypt. Zagazig J. Pharm. Sci., 22(1):17-25.

Adam, M.; Elhassan, G.O.M.; Yagi S.; Senol F.S.; Orhan, I.E.; Ahmed, A.A. and Efferth, T. (2018): In Vitro Antioxidant and Cytotoxic Activities of 18 Plants from the Erkowit Region, Eastern Sudan. Natural Products and Bioprospecting, 8:97–105. https://doi.org/10.1007/s13659-018-0155-0.

Adonova, M.; Urumova, V.; Petkova, B.; Slavov, B.; Dzhelebov, P.; Chaprazov, Ts.; Roydev, R. and Borissov, I. (2014): Haematological and biochemical parameters characterizing the progression of experimental Pseudomonas aeruginosa skin infection in dogs. Bulg. J. Vet. Med., 17 (1): 32 - 41.

Aekanurmaningdyah, A. and Kurniasih, (2018): Pathogenecity of Pseudomonas anguilliseptica Infection in Goldfish (Cyprinus Carpio). Int J cell Sci & mol biol., 4(5): pp. 00111-00116. DOI: 10.19080/IJCSMB. 2018.04.555650.

Afifi, A.A.M. (2016): Uses of ethanolic Malva sylvestris leaves extract as antimicrobial activity and immune stimulant in Oreochromis niloticus. Abbassa Int. J. Aqua., 9(2): 356-376.

Afiyanti, A.; Yuliani, M. and Handijatno, D. (2018): Leukocyte Count and Differential Leukocyte Count of Carp (Cyprinus carpio Linn) after Infected by Aeromonas salmonicida. In Proceedings of the 2nd International Conference Postgraduate School (ICPS 2018), pages 545-549. DOI: 10.5220/0007546705450549

Afolayan, AJ.; Aboyade, OM.; Adedapo, AA. and Sofidiya, MO. (2010): Antiinflammatory and analgesic activity of the methanol extract of Malva parviflora Linn (Malvaceae) in rats. Afr J Biotechnol., 9, 1225–1229.

Akbar, S.; Hanif, U.; Ali, J. and Ishtiaq, S. (2014): Pharmacognostic studies of stem, roots and leaves of Malva parvifloraL. Asian Pac J Trop Biomed., 4(5): 410-415.

Al- Qarawi, K.K. and Al-Obaidi, H.M.R. (2018): Detection of the active compounds in the leaves of the Common mallow plant Malva parviflora L. using GC-MS and HPLC technology. Kufa Journal for Agricultural Sciences, 10(4): 87-95.

Algammal, A.M.; Mabrok, M.; Sivaramasamy, E.; Youssef, F.M.; Atwa, M.H.; El-kholy, A.W.; Hetta, H.F. and Hozzein, W.N. (2020): Emerging MDR-Pseudomonas aeruginosa in fish commonly harbor oprL and toxA virulence genes and blaTEM, blaCTX-M, and tetA antibiotic-resistance genes. Scientific Reports, 10:15961. https://doi.org/10.1038/s41598-020-72264-4.

APHA (1998): A.P.H.A Standard Methods for the Examination of Water and Wastewater (20thed.), American Public Health Association, American Water Works Association and Water Environment Federation, Washington DC, USA.

Babaei Zarch, A.; Fallah Huseini, H.; Kianbakht, S.; Changaei, P.; Mirjalili, A. and Salehi, J. (2017): Malva sylvestris L. Protects from Fluoride Nephrotoxicity in Rat. Journal of Medicinal Plants, 16(61): 21- 32.

Barros, L.; Carvalho, AM. and Ferreira, IC. (2010): Leaves, flowers, immature fruits and leafy flowered stems of Malva sylvestris: a comparative study of the nutraceutical potential and composition. Food Chem. Toxicol., 48:1466–1472.

Batton, C.J. and Crouch, S.R. (1977): Spectrophotometric and kinetics investigation of the Berthelot reaction for the determination of ammonia, Analytical Chemistry, 49(3): pp. 464–469.

Beghdad, MC.; Benammar, C.; Bensalah, F.; Sabri, FZ.; Belarbi, M. and Chemat, F. (2015): Antioxidant activity, phenolic and flavonoid content in leaves, flowers, stems and seeds of mallow (Malva sylvestris L.) from North Western of Algeria. Afr. J. Biothecnol., 13: 486 - 91.

Beutler, E.; Duron, O. and Kelly, MB. J. (1963): Lab Clin. Med., 61, 882.

Bilen, S.; Filogh, A.M.O.; Ali, A.B.; Kenanoğlu, O.N. and Zoral, M.A. (2020): Effect of common mallow (Malva sylvestris) dietary supplementation on growth performance, digestive enzyme activities, haemotological and immune responses of common carp (Cyprinus carpio). Aquaculture International, 28:73–84. https://doi.org/10.1007/s10499-019-00444-9.

Bilen, S.; Kenanoglu, O.N.; Terzi, E.; Ozdemir, R.C. and Sonmez, A.Y. (2019): Effects of tetra (Cotinus coggygria) and common mallow (Malva sylvestris) plant extracts on growth performance and immune response in Gilthead Sea bream (Sparus aurata) and European Sea bass (Dicentrarchus labrax). Aquaculture 512, (2019) 734251. https://doi.org/10.1016/j.aquaculture.2019.734251.

Blaxhall, P.C. (1972): The haematological assessment of the health of freshwater fish. J Fish Biol 4: 593-604.

Blaxhall, PC. and Daisley, KW. (1973): Routine haematological methods for use with fish blood. J Fish Biol., 5(6): 771–781.

Bouriche, H.; Meziti, H.; Senator, A. and Arnhold, J. (2011): Anti-inflammatory, free radical-scavenging, and metal-chelating activities of Malva parviflora, Pharmaceutical Biology, 49(9): 942-946. https://doi.org/10.3109/13880209.2011.558102.

Bussolaro, D.; Filipak,F.;Ganrgioni, R.; Fernandes, L. and Ribeiro, C.A. (2008): The immune response of peritoneal macrophage due to exposure to inorganic lead in house mouse Musmusculus."Toxicology in Vitro, 22:254-260.

Capita, R. and Alonso-Calleja, C. (2013): Antibiotic-resistant bacteria: a challenge for the food industry, Critical Reviews in Food Science and Nutrition, 53(1):pp. 11–48.

Clinical and Laboratory Standards Institute (CLSI), (2015): Methods for Antimicrobial Dilution and Disk Susceptibility Testing of Infrequently Isolated or Fastidious Bacteria. 3rd ed.,

CLSI guideline M45, Wayne, Pa, USA.

Derwa, H.; AbdElWahab, M. and Kamal, K. (2017): Role of some biological pollutants in relation to disease occurrence in Oreochromis Niloticus. Suez Canal Vet. Med. J. SCVMJ22, 247–257.

Devakumar, D.; Jayanthi, J. and Ragunathan, M. (2013): Herbal alternate to Pseudomonas aerginosa infection in a freshwater crab, OZIOTELPHUSA SENEX SENEX. Int. J. Biol. Pharmacy Allied Sci. (IJBPAS).2, 2142–2147.

Diler, Ö.; Atabay, A. and Görmez, Ö. (2017): Bitkisel Katkı Maddesi Artemisia vulgaris' in Gökkuşağı Alabalıklarında (Oncorhyncus mykiss, Walbaum) Büyüme Performansı ve Antioksidan Aktivite GİRİŞ Antibiyotiklerin yem katkı maddesi olarak kullanımının gerek bakteriyel dirence yol açabileceği gere. Eğirdir Su Ürünleri Fakültesi Derg. 13, 119–131.

Dos Santos, A.C.; Sutili, F.J.; Heinzmann, B.M.; Cunha, M.A.; Brusque, I.C.M.; Baldisserotto, B. and Zeppenfeld, C.C. (2017): Aloysiatriphylla essential oil as additive in silver catfish diet: Blood response and resistance against Aeromonas hydrophila infection. Fish Shellfish Immunol., 62: 213-216.

Doumas, B.T.; Bayso, D.D.; Carter, R.J.; Peters, and Schaffer, R. (1981): Determination of total serum protein. Clin. Chem., 27:1642-1643.

Dugani, A.; Dakhil, B. and Treesh, S. (2016): Protective effect of the methanolic extract of Malva parviflora L. leaves on acetic acid-induced ulcerative colitis in rats. Saudi J Gastroenterol., 22(3): 226- 33.

Eissa, N.; El-Ghiet, E.; Shaheen, A. and Abbass, A. (2010): Characterization of Pseudomonas species isolated from tilapia "Oreochromis niloticus" in Qaroun and Wadi-El-Rayan lakes, Egypt. Global Veterinaria5, 116–121.

El Khoury, M.; Haykal, T.; Hodroj, M.H.; Abou Najem, S.; Sarkis, R.; Taleb, R.I. and Rizk, S. (2020): Malva pseudolavatera Leaf Extract Promotes ROS Induction Leading to Apoptosis in Acute Myeloid Leukemia Cells In Vitro. Cancers, 12, 435; doi:10.3390/ cancers12020435.

Eurell, T.E.; Lewis, S.D.H. and Grumbles, L.C. (1978): Comparison of selected diagnostic tests for detection of Motile Aeromonas Septicemia in fish. Am. J. Vol. Res., 39(8): 1384-1386.

European Medicines Agency (2019): List of references supporting the assessment report on Malva sylvestris L. and/or Malva neglecta Wallr., folium and Malva sylvestris L., flos. EMA/HMPC/749518/2016. 20 November 2018, Committee on Herbal Medicinal Products (HMPC). An agency of the European Union, pp. 1-39. www.ema.europa.eu/contact.

Farhan, H.; Rammal, H.; Hijazi, A.; Hamad, H.; Daher, A.; Reda, M. and Badran, B. (2012): In vitro antioxidant activity of ethanolic and aqueous extracts from crude Malva parviflora L. grown in Lebanon. Asian J Pharm Clin Res., 5(Suppl 3):234-8.

Farhan, A.S. and Mohammed, M.T. (2020): Histopathological changes in kidney and liver with oxidative stress and protection by plant extracts. Veterinarija ir Zootechnika, 78(100):50-6.

Gasparetto, J.C.; FerreiraMartins,C.A.; Hayashi,S.S.;Otuky,M.F. and Pontarolo, R. (2011): Ethnobotanical and scientific aspects of Malva sylvestris L.: a millennial herbal medicine. J Pharm Pharmacol., 64: 172–189.

Golas, I.; szmyt, M.; Potorski, J.; Lopata, M.; Gotkowska-Plachta, A. and Glinska-Lewczuk, K. (2019): Distribution of Pseudomonas fluorescens and Aeromonas hydrophila Bacteria in a Recirculating Aquaculture System during Farming of European Grayling (Thymallus

thymallus L.) Broodstock. Water (11): 376.

Hajyani, S. and Modaresi, M. (2016): The effect of Malva sylvestris extract on blood protein and gamma interferon of Candida albicans infected mice. J Chem Pharm Res., 8(4):1277-1281.

Hardi, E.H.; Nugroho, R.A.; Kusuma, I.W.; Suwinarti, W.; Sudaryono, A. and Rostika, R. (2019): Borneo herbal plant extracts as a natural medication for prophylaxis and treatment of Aeromonas hydrophila and Pseudomonas fluorescens infection in tilapia (Oreochromis niloticus) [version 2; peer review: 2 approved, 1 approved with reservations] F1000Research, 7:1847. https://doi.org/10.12688/f1000research.16902.2.

Havixbeck, J.J.; Rieger, A.M.;Wong, M.E.; Hodgkinson, J.W. and Barreda, D.R. (2016): Neutrophil contributions to the induction and regulation of the acute inflammatory response in teleost fish. J. Leukoc. Biol., 99, 241–252.

Houot, O.(1985): Interpretation of Clinical Laboratory Tests, edited by G. Siest, J. Henny, F. Schiele and D. S. Young, BiochemicalPublications.

Hrubec, T.C.; Cardinale, J.L. and Smith, S.A. (2000): Hematology and Plasma Chemistry Reference Intervals for Cultured Tilapia (Oreochromis Hybrid).Vet Clin Pathol., 29(1): 7-12.

Hussain, L.; Ikram, J.; Rehman, K.; Tariq, M.; Ibrahim, M. and Akash, MSH. (2014): Hepatoprotective effects of Malva sylvestris L. against paracetamol-induced hepatotoxicity. Turk. J. Biol., 38(3): 396 - 402.

Innes, W.T. (1966): Exotic aquarium fishes. 19th. ED. Aquarium incorporated. New Jersey, USA.

Jorgensen, J.H.; Turnidge, J.D. and Washington, J.A. (1999): Antibacterial Susceptibility Tests: Dilution and Disk Diffusion Methods. In: Murray, P.R., Barron, E.J., Praller, M.A., Tenover, F.C. and Yolken, R.H., Eds. Manual of Clinical Microbiology. Washington, D.C., ASM Press, pp. 1526-1562.

Khalil, R.H.; Khalil, S.A.; Saad, T.T. and Safaa, M.H. (2010): Studies on Pseudomonas Septicemia among Cultured Oreochromus niloticus. Journal of the Arabian Aquaculture Society, 5(1):55-63.

Klūga, A.; Terentjeva, M.; Kántor, A.; Kluz, M.; Puchalski, C. and Kačániová, M. (2017): Antibacterial Activity of Melissa officinalis L., Mentha piperita L., Origanum vulgare L. and Malva mauritiana against Bacterial Microflora Isolated from Fish. Advanced Research In Life Sciences, 1(1), 75-80. DOI: 10.1515/arls-2017-0013.

Kuebutornye, F.K.A. and Abarike, E.D. (2020): The contribution of medicinal plants to tilapia aquaculture: a review. Aquaculture international (28): 965-983.

Lagunas-Herrera, H.; Tortoriello, J.; Herrera-Ruiz, M.; Martínez-Hernández, GB.; Zamilpa, A.; Aguilar, L.; García, M.; Lombardo-Hearl, G. and Jiménez- Ferrer, E. (2019): Acute and chronic antihypertensive effect of fractions, tiliroside and scopoletin from Malva parviflora. Biol Pharm Bull 42:18–25 https://www.ncbi.nlm.nih.gov/pubmed/30606987.

Lamas, J.; Santos, Y.; Bruno, D.W.; Toranzo, A.E. and Anado´,N.R. (1994): Non-specific cellular responses of rainbow trout to Vibrio anguillarum and its extracelluar products (ECPs). Journal of Fish Biology 45, 839–854.

Magdy, I.; El-Hady, M.; Ahmed, H.; Elmeadawy, S. and Kenwy, A. (2014): A contribution on Pseudomonas aeruginosa infection in African catfish (Clarias gariepinus). Res. J. Pharm. Biol. Chem. Sci. 5, 575–588.

Marouane, W.; Soussi, A.; Murat,JC.; Bezzine, S. and El Feki, A. (2011): The protective effect of Malva Sylvestris on rat kidney damaged by vanadium. Lipids Health Dis., 10: 65.

Martins, C.A.F.; Campos, M.L.; Irioda, A.C.; Stremel, D.P.; Trindade,

A.C.L.B. and Pontarolo, R. (2017): Anti-Inflammatory effect of Malva sylvestris, Sida cordifolia, and Pelargonium graveolens is related to inhibition of prostanoid production. Molecules 2017, 22, 1883; doi: 10.3390/molecules22111883.

Mazrouh, M.M.; Amin, E.M.; Hegazi, M.A.; Hussien, K.A. and Attia, Z.I. (2015): Some Haematological Parameters and Blood Picture of Oreochromis niloticus in Manzalah Lake, Egypt. IOSR Journal of Environmental Science, Toxicology and Food Technology (IOSR-JESTFT) e-ISSN: 2319-2402,p- ISSN: 2319-2399. 9(5): Ver. I, PP 11-19. www.iosrjournals.org.

Mihaylova, D.; Popova, A.; Denkova, R.; Alexieva, I. and Krastanov, A. (2014): In vitro antioxidant and antimicrobial activity of extracts of bulgarian Malva sylvestris L. Conference: First National Conference of Biotechnology, Sofia, 100, 41-48.

Municio, C.; Alvarez, Y.; Montero, O.; Hugo, E.; Rodríguez, M.; Domingo, E.; Alonso, S.; Fernández, N. and Crespo, M.S. (2013): The Response of Human Macrophages to β-Glucans Depends on the Inflammatory M,ilieu. PLOS ONE, 8(4).

Nabavi, SF.; Moghaddam,AH.; Eslami, S. and Nabavi, SM. (2012): Protective effects of curcumin against sodium fluoride-induced toxicity in rat kidneys. Biol. Trace. Elem. Res., 145: 369 - 74.

Nishikimi, M.; Roa, N.A. and Yogi, K. (1972): The occurrence of superoxide anion in the reaction of reduced phenazine methosulphate and molecular oxygen. Biochem. Bioph. Res. Common., 46, 849 – 854.

Ododo, M.M.; Choudhury, M.K. and Dekebo, A.H. (2016): Structure elucidation of β-sitosterol with antibacterial activity from the root bark of Malva parviflora. SpringerPlus., 5:1210. DOI 10.1186/s40064-016-2894-x.

Paul, D. (2016): "A review on biological activities of common mallow (Malva sylvestris L.)," Innovare Journal of Life Sciences, 4(5): pp. 1–5.

Perez Gutierrez, RM. (2012): Evaluation of hypoglycemic activity of the leaves of Malva parviflora in streptozotocin-induced diabetic rats. Food Funct., 3:420-7.

Petrie, A. and Watson, P. (1999): Statistics for veterinary and animal science. 1st Ed., p. 90-99, 110-115, The Blackwell Science Ltd, United Kingdom.

Rackova, L.; Drabikova, K.; Jancinova, V.; Perecko, T.; Smidrka, J.; Harmatha, J.; Toth, J.; Kostalova, D.; Bezek, S.; Stefe, M. and Nosal, R. (2009): Structural aspects of antioxidant action of selected natural. Free Rad. Res., 43: S27-97.

Rajaraman, V.; Nonnecke, B.J.; Franklin, S.T.; Hamell, D.C. and Horst, R.L. (1998): Effect of vitamin A and E on nitric oxide production by blood mononuclear leukocytes from neonatal calves fed milk replacer. J.Dairy Science, 81: 3278-3285.

Raman, R.P. (2017): Applicability, feasibility and efficacy of Phytotherapy in Aquatic Animal Health Management. American Journal of Plant Sciences, (8): 257-287.

Ramírez-Serrano, CE.; Jiménez-Ferrer, E.; Herrera-Ruiz, M.; Zamilpa, A.; Vargas-Villa, G.; Ramírez, RJ.; Chavarría, A.; Tortoriello, J.; Pedraza, G. and Perez-Martínez, L. (2019): A Malva parviflora's fraction prevents the deleterious effects resulting from neuroinflammation. Biomed Pharmacother 118:109349.

Rasheed,HU.; Nawaz, H.; Rehman, R.; Mushtaq, A.; Khan, S. and Azeem, MW. (2017): Little Mallow: A review of botany, composition, uses and biological potentials. International journal of chemical and biochemical sciences, (12): 157-161.

Rashidian, G.; Kimia, K.; Prokic,M. and Caterina, F. (2019): Extract of

common mallow(Malvae sylvestris) enhances growth, immunity, and resistance of rainbow trout (Oncorhynchus mykiss) fingerlings against Yersinia ruckeri infection. Fish and fish immunology (96).

Razavi, SM.; Zarrini, G.; Molavi, G. and Ghasemi, G. (2011): Bioactivity of Malva sylvestris L., a medicinal plant from Iran. Iran J Basic Med Sci., 14(6):574-579.

Reinhold, R.R. (1953): Determination of serum albumin. Clin. Chem., 21: 1370 - 1372.

Reitman, S.and Frankel, S. (1957): A colorimetric method for determination of serum Glutamic oxaloacetic transaminase and serum Glutamic pyruvic transaminase. Am J. clin. Path., 25-65.

Reverter, M.; Bontemps, N.; Lecchini, D.; Banaigs, B. and Sasal, P. (2014): Use of plant extracts in fish aquaculture as an alternative to chemotherapy: current status and future perspectives. Aquaculture 433:50–61.

Saad, AB.; Rjeibi, I.; Alimi, H.; Ncib, S.; Smida, A.; Zouari, N. and Zourgui, L. (2017): Lithium induced, oxidative stress and related damages in testes and heart in male rats: the protective effects of Malva sylvestris extract. Biomed Pharmacother 86:127–135.

Saad, T.T.; Sara, A.A. Ketkat andFardos, A. Mohammed (2014): Changes Associated with Pseudomonas Infection in Cultured Orechromis Species and its Relations to Economic Losses of Fish Production Farms. Am. J. Life. Sci. Res, 238 – 252.

Saxena,MJ.; Sexana, R.; Nema, D.; Sing, A. and Gupta, A. (2013): Phytochemistry of medicinal plants. J Pharm Phytochem.,1(6): 168-182.

Schltz, L.A. (1987): "Veterinary Haematology". 3rd ed., Lea and Febiger., 39(2): 217-222.

Shadid, K.A.; Shakya, A.K.; Naik, R.R.; Jaradat, N.; Farah, H.S.; Shalan, N.; Khalaf, N.A. and Oriquat G.A. (2021): Phenolic Content and Antioxidant and Antimicrobial Activities of Malva sylvestris L., Malva oxyloba Boiss., Malva parviflora L., and Malva aegyptia L. Leaves Extract. Journal of Chemistry, Hindawi, Volume 2021, Article ID 8867400, 10 pages. https://doi.org/10.1155/2021/8867400.

Shale TL.; Stirk, WA. andVan Staden, J. (2005): Variation in antibacterial and anti-inflammatory activity of different growth forms of Malva parviflora and evidence for synergism of the anti-inflammatory compounds. J Ethnopharmacol, 96, 325–330.

Singh, A. and Navneet (2017): Ethnomedicinal, Antimicrobial and Pharmacological aspects of Malva parviflora Linn.; A review. The Journal of Phytopharmacology, 6(4): 247-250.

Stoskopf, MK. (1993): Clinical pathology. In: Stoskopf MK, ed. Fish Medicine. Philadelphia, Pa: WB Saunders, 113-131.

Tabaraki, R.; Yosefi, Z. and Asadi, G.H.A. (2012): "Chemical composition and antioxidant properties of Malva sylvestris L," Journal of Research in Agricultural Sciences, 8, 59–68.

Terech-Majewska, E. (2016): Improving disease prevention and treatment in controlled fish culture. Arch. Pol. Fish. (24): 115–165.

Terzi, E. and Isler, H. (2019): Antibiotic resistance genes of Escherichia coli in coastal marine environment of Eastern Black Sea, Turkey. In: Fresenius Environmental Bulletin. 28. pp. 1594–1601.

Tietz, N.W. (1995): Clinical Guide to Laboratory Tests, WB Saunders, Philadelphia, Pa, USA, 3rd edition, 1995.

Vallejos-Vidal E, Reyes-Lopez, F.; Teles, M. and MacKenzie, S. (2016): The response of fish to immunostimulant diets, Fish and Shellfish Immunology, 56, pp. 34-69. DOI: 10.1016/j.fsi.2016. 06.028.

Yagi, K. (1984): Lipid peroxidation in blood plasma or serum. Meth.Enzymol. 105:328-331.

Yang, R.; Yu, L.; Zeng, H.; Liang, R.; Chen, X. and Qu, L. (2012): The interaction of flavonoid-lysozyme and the relationship between molecular structure of flavonoids and their binding activity to lysozyme. J Fluoresc 22:1449–1459.

Yin, X.; Li, Z.; Yang, K.; Lin, H. and Guo, Z. (2014): Effect of guava leaves on growth and the non-specific immune response of Penaeus monodon. Fish & Shellfish Immunology, 40, 190–196. https://doi.org/10.1016/j.fsi.2014.07.001.

Zare, P.; Mahmoudi, R.; Shadfar, S.; Ehsani, A.; Afrazeh, Y. and Saeedan,A. (2012): Efficacy of chloroform, ethanol and water extracts of medicinal plants, Malva sylvestris and Malva neglecta on some bacterial and fungal contaminants of wound infections. J Med Plants Res., 6(29): 4550-4552.

Zexia, G.; Weimin, W.; Yi, Y.; Abbas, K.; Dapeng, L.; Guiwei, Z. and Diana, J.S. (2007): Fish Physiol Biochem., 33: 213–222.DOI 10.1007/s10695-007-9133-x.

Zhang, Z.; Zhou, B.; Wang, H.; Wang, F.; Song, Y.; Liu, S. and Xi, S. (2014): Maize purple plant pigment protects against fluoride-induced oxidative damage of liver and kidney in rats. Int. J. Environ. Res. Public Health, 11: 1020 - 33.

محاولة استخدام بدائل المضادات الحيوية لرفع الكفاءة المناعية وأداء النمو في البلطي النيلي

ايناس أحمد ابراهيم خريص ، ايمان محمد السعيد الشربينى

E-mail: **myvetaddress@gmail.com** Assiut University web-site: www.aun.edu.eg

هذه الدراسه تكشف معمليا التأثير المضاد للبكتريا للمستخلص الإيثانولي من أوراق نبات الخبيزة (***Malva parviflora***) ضد ميكروب السيدوموناس فلوريسينس (***Pseudomonas fluorescens***), (قياس أقل تركيز مثبط **(MIC)** وأقل تركيز قاتل **(MBC)**), معرفة المكونات البيولوجية المسئولة عن كفاءته باستخدام تحليل جهاز **Gas-Chromatography- Mass-Spectrometer-(GC-MS)** بجانب تقييم تأثيره داخل الجسم علي أسماك البلطي النيلي كنبات طبي لمقاومة العدوي بميكروب السيدوموناس فلوريسينس. تم تقسيم عدد 225 سمكة إلي 5 مجاميع: مجموعة (1): المجموعة الضابطة السلبية, مجموعة (2): المجموعة الضابطة الإيجابية تم عدواها عند اليوم الستين بجرعة **($1x10^3$ CFU/0.2ml)** بالحقن فى العضل ,مجموعة (3), (4) و (5): تم تغذيتها لمدة 60 يوم بالمستخلص بجرعات (0.25- 0.5 و1 جرام/كجم عليقه) علي التوالي. عينات الدم تم تجميعها عند اليوم 30, 60 وبعد العدوى. أظهرت نتائج اختبار الحساسية حساسية ميكروب السيدوموناس فلوريسينس للمستخلص. نتائج **MIC** و**MBC** كانت 3.90 و 31.25 ملجم/مليلتر علي التوالي. الأوزان ومستوي النيترك أوكسيد للخلايا الأكولة ونشاط الليسوزوم زادوا معنوياً في كل المجاميع المعالجة عن الكنترول وخاصة المجموعة (4) المعالجة (0.5 جرام/كجم) في حين زادت مجموعتين (3) و (4) من معدل الاحياء الي 80% مقارنة ب 40% للمجموعة (2) الضابطة الإيجابية. العد الكلي لخلايا الدم البيضاء والليمفاوية ووحيدة الخلية زادوا معنوياً في كل المجاميع المعالجة عن المجموعة (1) الضابطة السلبية طوال مدة التجربة. إنزيمات **(AST, ALT, ALP)** واليوريا والكرياتينين لم يظهروا أي تغيير معنوي بين المجموعتين (1) و (3) بينما زادوا في مجموعتي (4) و (5) بزياده جرعه المستخلص. البروتين الكلى و الألبيومين والجلوبيولين لم يظهروا أي تغيير معنوي بين المجاميع. مستوى **(MDA)** أظهر نقصا معنويا في المجموعة (3) عن المجموعة (1) و بعد العدوي أظهرت كل المجاميع المعالجة نقصا معنويا عن المجموعة (2). مضادات الأكسدة **(SOD** و**GSH)** زادوا معنوياً في كل المجاميع المعالجة عن الكنترول بدرجات مختلفة. في النهاية, أدى المستخلص المستخدم إلى زيادة معدلات الأداء والمناعة ومضادات الاكسدة. كانت الأثار الجانبية للمستخلص على وظائف الكبد والكلية مرتبطة بزيادة الجرعة لذلك لم تسجل جرعة 0.25 جرام/كجم أي أثار جانبيه بينما سجلت جرعة 0.5 جرام/كجم أعلى وزن مكتسب.