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New Insights into the Effectiveness of Fungi-Degraded Date Pits Supplemented Diet on the Physiological and Biological Indices of *Oreochromis niloticus* Fingerlings

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ABSTRACT

This study examined the effects of fungi- degraded date pit (FDDP) as a dietary supplement in fish diets *in vitro* and *in vivo*. In the *in vitro* study, FDDP was prepared and characterized for its nutritional and biological impacts on animal health. A total of nine Nile tilapia (*Oreochromis niloticus*) experimental fish groups were used to assess the sub-chronic toxicity of FDDP crude and ethanolic extract on growth performance and biochemical markers. Four groups of fish (15±0.1g) received varying concentrations of crude FDDP (5, 10, 20, 30g/ kg diet), while another four received varying concentrations of FDDP ethanolic extract (2.5,5,7.5,10 g/kg diet) in addition to the control group. The chemical compositions of FDDP showed high protein, fiber, organic matter, and hemicellulose contents. For the analysis of FDDP, an increase was recorded in mineral contents and xylanase & cellulase enzyme activities, compared to the degraded date pits (DDP). The results of fish biochemical composition and physiological analyses showed that diets containing ethanolic FDDP extract (1%) and crude FDDP (2%) had the highest weight gain (WG), condition factor (K) and protein efficiency ratio (PER) values. Additionally, an increase was detected in the antioxidant capacity by upregulating reduced glutathione (GSH) levels and superoxide dismutase (SOD) activities. In both liver and brain tissues, oxidative stress levels, such as thiobarbituric acid reactive substances (TBARS) and nitric oxide (NO) were declined. In conclusion, our findings indicate that supplementing fish diets with either FDDP ethanolic extract (1%) or crude FDDP (2%) could promote fish growth, increase physiological competence and stress resistance while reducing oxidative stress in the Nile tilapia.

INTRODUCTION

Food loss and waste reduction are important ways to reduce production costs and increase the efficiency of the global food system, as well as contributing to environmental sustainability. As a result from an environmental standpoint, reducing food loss and

waste is expected to have several positive effects, such as improving water management, climate change, marine resources, terrestrial ecosystems, forestry, and biodiversity, among other things.

Several studies have demonstrated that, phytogenic feed additives in fish diets can promote growth, anti-stress, appetite stimulation, hematological and biochemical status in addition to disease resistance **(Abo-State *et al*., 2017; Bharathi *et al*., 2019)**. Furthermore, since many fish producers cannot afford adding expensive feed additives to fish diets **(Hass *et al*., 2016)**; nowadays, the use of organic feed additives to improve feed use efficiency in aquaculture has become a critical demand **(Tibbetts *et al*., 2017**).

Phoenix dactylifera L. is a major fruit crop in the Middle East and the Mediterranean region. According to the Food and Agriculture Organization **(FAO, 2011)**, Egypt produces 1330,000 ton per year, ranking the second to China. Egypt's dates accounted for roughly 17% of global production. Date seeds (date pits) make up 10- 20% of the date fruit. Date pits are rich in protein, fat, dietary fiber and phenolic acids **(Attia *et al*., 2021)**. In addition, they can be used to boost the nutritional value of food products **(Guizani *et al*., 2014; Tafti *et al*., 2017)**.

Date pits are currently used as a feed additive for sheep **(Aldosari *et al*., 1995)**, broilers **(Alyileili *et al*., 2020)** and fish **(Dawood *et al*., 2020)**. Date pits are rich in bioactive non-nutrient compounds called phytochemicals, a secondary plant metabolite in addition to nutrient-rich compounds **(Bouhlali *et al*., 2020)**.

Date pits contain high amounts of crude protein, fiber, neutral detergent fiber (NDF) and acid detergent fiber (ADF) **(Rahman *et al*., 2007)**, which limit their use as a feed ingredient. Fish (animals) can better use date pits if specific enzymes degrade these fibers into simpler carbon molecules. Xylanase enzymes are used in food formulation to hydrolyze or release nutrients blocked by non-degradable cellulose and hemicellulose **(Leisola *et al*., 2002)**. This increases food consumption while decreasing the amount of animal feces produced. The degradation of dietary fiber by microorganisms producing xylanase enzymes is cheaper than using enzymes in animal feed. *Trichoderma reesei* (also known as *Hypocrea jecorina*) is the most studied cellulolytic fungi **(Foreman *et al*., 2003)**. The U.S. Food and Drug Administration has approved using it for animal (fish) feed **(Food & Drug Administration (FDA), 1993)**. It efficiently produces xylanases and cellulase-degrading enzymes in large quantities **(Fang *et al*., 2021)**. Additionally, the degradation of date pits improves the chemical constituents and produces bioactive substances with added value for animal nutrition (**Alyileili *et al*. (2020)**.

The present study aimed to produce a safe, low-cost byproduct with high biological impact as a supplement in fish diets, focusing on the investigation of its biochemical and physiological effects on *Oreochromis niloticus*.

MATERIALS AND METHODS

2. Materials and methods of the study

2.1. Degraded date pits (DDP)

To avoid sticking dirt, the date pits (Phoenix dactylifera L.) were purchased from El-Wadi El Gedid Factory for Packaging Dates (El-Wadi El Gedid, Egypt). They were washed with regular tap water and dried for 8 hours at 80˚C. Date pits were finely ground using a feed grinder, sieved through 0.6mm sieves and stored at –20˚C until used. *Trichoderma reesei* fungi (AUMC 5829) were purchased from Assiut University Mycological Center AUMC, Assuit, Egypt. Half of the grinded date pits was degraded. The chemical and nutritional compositions of both DDP and FDDP were determined prior to the preparation of the test diets (Table 1).

2.2. Trichoderma reesei- fungi culture

Ampoules of dried (vacuum) *Trichoderma reesei* fungi culture (AUMC 5829) were rehydrated with 25ml of potato dextrose agar media (PDA; potato infusion 200g, agar 20g, and dextrose 20g in 1L distilled water, with pH value of 5.6±0.2, then a sub-culture of *Trichoderma reesei* fungi was transferred to grow on PDA plates at 30˚C for seven days in the dark.

2.3. Production of fungi- degraded date pits (FDDP)

For solid state fermentation (SSF), autoclavable bags were used to produce fungi-degraded date pits. Initial cleaning and sterilization of glassware as well as substrating DDP at 12˚C for 20 min were performed, followed by media (one liter of culture media contained 1% malt extract, 1.89 mM $(NH_4)_2SO_4$, 10.29 mM KH_2PO_4, 18.02 mM $CaCl_2$, 1.21mM $MgSO_4.7H_2O$, 35.29 mM $NaNO_3$, 6.70 mM KCl, 2.01 µM $CuSO_4.5H_2O$, 1.79 µM $FeSO_4.7H_2O$, 7.17 µM $MnSO_4.4H_2O$, 4.86 µM $ZnSO_4.7H_2O$, 84.05 µM $CoCl_2.6H_2O$ and 0.1% tween 80 (v/v), pH 5.5 and 2.5% lactose). Next, eight 6mm agar plugs from *Trichoderma reesei* active margins were inoculated with half a kilogram of degraded date pits (DDP). The autoclavable bags were incubated at 28˚C for two weeks. Since the process was mostly aerobic, constant aeration was required. The fungi degraded date pits (FDDP) were autoclaved twice and stored at –20˚C. A series of tests confirmed the formation of FDDP and the action of *Trichoderma reesei* fungi over DDP.

2.4. Determination of chemical compositions of samples

Samples (DDP and FDDP) were analyzed for contents of crude protein (CP), ash, crude fat, NDF, acid detergent fiber (ADF) and acid detergent lignin (ADL). Dry matter (DM) was determined after drying at 105˚C and ash after combustion at 550˚C. Crude fat was extracted for 6h with petroleum ether; whereas, the Kjeldahl method was used to

determine nitrogen (N) (AOAC, 1990). CP was calculated as N × 6.25. NDF, ADF, and ADL were determined according to the methods of **Van Soest *et al*. (1991)**, using an ANKOM 220 fiber analyzer (ANKOM Technology Corporation, NY, USA). Hemicellulose was calculated as NDF – ADF and cellulose was evaluated as ADF – ADL **(Rinne *et al*., 1997)**.

2.5. Sample preparation for mineral analysis

The samples (DDP and FDDP) were analyzed in triplicate for the macro and micro-elements as nitrogen (N), phosphorous (P), potassium (K), calcium (Ca), magnesium (Mg), iron (Fe), zinc (Zn), manganese (Mn), copper (Cu), using a NOUVA400 atomic absorption spectrophotometer (Analytik Jena, Germany) **(American Public Health Association [APHA] *et al*., 1999)**.

2.6. Morphological characterization

In this study, scanning electron microscope (SEM) was utilized to examine the surface morphology of degraded date pits before and after fermentation with *Trichoderma reesei* fungi. Gold sputtering was used to ensure surface conductivity. The SEM conditions for secondary electrons used were: 15 kV beam intensity acquiring 1,500 X magnification **(Kalantar-zadeh & Fry, 2008)**.

2.7. Crude enzymes extraction

An enzyme extraction from FDDP was compared to DDP after 5 days, 1 week and 2 weeks of inoculation. An hour was needed to extract enzymes from 10g of powder in a 50ml buffer solution (Citrate phosphate, 0.1 M buffer; pH 5.0). The extract was filtered through damp cheesecloth and centrifuged at 805xg for 15min to obtain extracellular enzymes **(Shamala & Sreekantiah, 1987)**. Cellulase was converted to cellulose and then to glucose **(King & Vessal, 1969)**. The released glucose forms a red complex with dinitrosalicyclic acid (DNS) at 575nm **(Miller, 1959)**.

2.8. In vitro analyses of ethanolic and aqueous extracts of DDP and FDDP

2.8.1. Preparation of aqueous and ethanolic extracts. Each sample (1g) was extracted twice by stirring with 30ml of 70% ethanol. For the aqueous extract, a decoction was prepared by boiling 100g of DDP and FDDP powder in one liter of distilled water for 15min, and then the mixture was filtered, lyophilized and stored at –20˚C for further analysis.

2.8.2. Phytochemical investigations of DDP and FDDP extracts. Total phenolics content of DDP and FDDP aqueous and ethanolic extracts were spectrophotometrically assessed following the Folin–Ciocalteu method **(Slinkard & Singleton, 1977)**. Total flavonoid was analyzed by aluminum chloride colorimetric method **(Chang *et al*., 2002)**.

2.8.3. *In vitro* biological screening. The 2,2-diphenyl-1-picryl hydrazyl (DPPH) scavenging activity was assessed according to the study of **Blois (1958)**. Further, the nitric oxide (NO) radical scavenging activity was evaluated according to **Garrat (1964)**.

2.8.4. Determination of cellular cytotoxicity of DDP/FDDP extracts. To begin with, a sterile heparin tube was used to isolate and culture human white blood cells (WBCs). "N" represents number of viable or nonviable cells and "D" represents sample dilution (1:1 with the trypan blue). For using the cells for testing, at least 90% must be viable. Then, different extracts were tested on WBCs in 96-well plates with 100µL of cell suspension (1×10^5cells/ ml). The plate was incubated at 37˚C for 24h, with 5% humidified CO_2. After collecting a semi-conflict cell layer, an amount of 100µL of various DDP and FDDP extracts was applied (12.5, 25.50, 100, 200 g/ml), and media (control) were incubated for three days at 37˚C. Afterward, each cell layer received 100µL of working solution (40µg/ ml) and incubated for three hours at 37˚C in 5% CO_2. Hundred microliters of fixation solution (0.5% formalin with 1% calcium chloride) was applied for one minute and 0.5% formalin, with 1% calcium chloride fixation for one min, followed by 5min in a destaining solution (50% ethanol with 1% glacial acetic acid). Assuring a percentage>85 of cell viability was determined by measuring stain intensity at 540nm, using ELISA microplate reader. The percent of cell viability= AT/AC ×100; where, AT: the mean absorption of the treatment cells at various concentrations of each extract and [AC = the mean of absorbance of control untreated cells only with culture medium] **(Repetto *et al*., 2008)**.

***2*.8.5. Quantitative determination of DDP and FDDP ethanolic fractions contents by high performance liquid chromatography (HPLC).** The analysis of the ethanolic fractions of DDP and FDDP extracts were carried out on an Agilent Technologies 1100 HPLC system fitted with an auto sampler and diode array detector. The analytical column was Eclipse XDB-C18, with a C18 guide (Phenomenex, Torrance, CA) column (150 X 4.6µm; 5µm). The mobile phase was acetonitrile (solvent A) acetic acid (v /v) of 2% (solvent B). The flow rate was held at 0.8ml/ min for 60min, with the gradient set to: 100% B to 85% B in 30min, 85% B to 50% B in 20min, 50% B to 0% B in 5min, and 0% B to 100% B in 5min. The amount of injections was 50µL, and the peaks were simultaneously tracked for benzoic acid and cinnamic acid derivatives at 280 and 320nm, respectively, in addition to flavonoids at 360nm. DDP and FDDP fractions identification was made by comparing the relative retention times of sample peaks with those of the reference standards **(Gerber *et al*., 2004)**.

2.9. Experimental diets

The present study evaluated nine isonitrogenous (29%, CP), isoenergetic (18 MJ/kg) experimental diets for the Nile tilapia (*Oreochromics niloticus*) fingerlings (Table 1). The experiment was designed as follows: a control basal diet (D0), four isonitrogenous-isoenergetic diets containing graded concentrations of FDDP ethanolic extract as a feed

additive (2.5, 5, 7.5, 10g of FDDP/ kg diet), which were designated as D1, D2, D3, and D4, and four isonitrogenous-isoenergetic diets containing different concentrations of crude FDDP as a feed supplement (5, 10, 20, 30g of FDDP/ kg diet). All dry ingredients were grinded, screened, and mixed with a mixer. The oil was slowly added to the vitamins and minerals mixture with a continuous mixing and then added to the ingredients. The warm water was slowly added to form dough, which was then minced into pellets. Pellets were dried in a forced air-drying oven at 60˚C for 24h. They were kept in dry, sealed bags at 20˚C until needed. The test diets' biochemical composition was determined in triplicate using standard methods **(AOAC, 1995)**.

Table 1. Ingredients and proximate composition (% DM) of control diet and the two forms of FDDP supplemented diets fed to the Nile tilapia (*Oreochromis niloticus)*

Ingredient	D0	D1	D2	D3	D4	D5	D6	D7	D8
Fish meal[1]	150	150	150	150	150	150	150	150	**150**
Soybean meal[2]	350	350	350	350	350	350	350	350	**350**
Yellow corn	100	100	100	100	100	100	100	100	**100**
Wheat bran	170	170	170	170	170	170	170	170	**170**
Wheat flour	150	1 47.5	145	142.5	140	145	140	130	**120**
Sunflower oil	50	50	50	50	50	50	50	50	**50**
Vitamins& minerals Premix[3]	30	30	30	30	30	30	30	30	**30**
FDDP extract	**0**	**2.5**	**5**	**7.5**	**10**	**-**	**-**	**-**	**-**
FDDP crude	**-**	**-**	**-**	**-**	**-**	**5**	**10**	**20**	**30**
Biochemical composition (% DM)									
Dry matter	0.48	91.00	39.90	90.89	90.59	90.99	89.99	89.89	**90.11**
Crude protein	8.89	29.50	29.90	29.34	29.99	30.05	29.88	29.74	**29.89**
Lipid	7.97	8.30	8.22	7.99	8.79	8.20	8.12	7.79	**7.97**
Ash	7.2	7.00	6.50	7.10	6.29	6.99	6.39	7.15	**7.25**
Fiber	3.66	3.70	3.90	3.85	3.49	3.65	3.90	3.63	**3.06**
NFE[4]	2.26	50.50	51.48	51.72	51.44	51.11	51.71	51.69	**51.83**
GE (MJ/Kg)[5]	**8.34**	**19.16**	**19.20**	**18.98**	**19.40**	**19.12**	**19.20**	**18.99**	**19.12**

[1]lab made (68% protein),[2](42% protein),[3]Vitamins and minerals premix (mg kg^{-1}): p-amino benzoic acid (9.48); D-biotin (0.38); inositol (379.20); niacin (37.92); Ca pantothenate (56.88); pyridoxine HCl (11.38); riboflavin (7.58); thiamine HCl (3.79); L-ascorbyl-2-phosphate Mg (APM) (296.00); folic acid (0.76); cyanocobalamine (0.08); menadione (3.80), vitamin A palmitate (17.85); α-tocopherol (18.96); calciferol (1.14). K_2PO_4 (2.011); $Ca_3(PO_4)_2$ (2.736); Mg $SO_4 7H_2O$ (3.058); NaH_2PO_4 $2H_2O$ (0.795).4Nitrogen-free extracts (NFE) = 100 – [% ash + % lipid + % protein + % fiber]. [5]Gross energy was quantified based on 23.6, 39.5 and 17.2 kJ/g for protein, lipid and carbohydrates, respectively **(NRC, 1993)**.

2.10. Fish and experimental design

Five hundred Nile tilapia (*Oreochromics niloticus*) fish were purchased from a private farm and transported to the lab. For adaptation, fish were placed in $3m^3$ fiberglass tank and fed on commercial diet for a week (30% crude protein and 8% lipid, Aller Aqua, Egypt). Nine dietary fish groups were identified in triplicate and randomly distributed

into 27 (200L) aquariums, with a stocking density of 15 fish per aquarium and an initial body weight of 15g/ fish.

The culture tanks were provided with air stones and about 30% of their water was replaced daily with similar-temperature fresh water. Throughout the trial, the culture unit was lit at 12:12 12:12-h light: dark cycle. Water quality parameters, including temperature, pH and dissolved oxygen were weekly measured, using a Hanna HI-9828 portable water quality meter, while salinity was measured using a Hanna GG-201/211 portable refractometer. Fish were hand- fed three times per day with 4% of fish body weight at 08:00am, 13:00pm and 17:00pm; whereas, the uneaten food was siphoned and quantified after each meal. Upon completing the feeding trial, each fish tank was netted, counted, and weighed collectively. Ten fish from each tank were rinsed with distilled water, dried with a paper towel, and their weights (g) and lengths (cm) were measured. The remaining fish were frozen at –20˚C for further analyses.

2.11. Evaluation of growth performance, feed utilization and biometric indices

Fish growth performance and feed utilization were analyzed in terms of survival (S%), weight gain (WG, g), percentage weight gain (%WG), specific growth rate (SGR, %), feed conversion ratio (FCR) and protein efficiency ratio (PER). The following formulae were used: Survival (S%) = (final fish count/ initial fish count) × 100, WG (g) = final fish weight (FW) (g) – initial fish weight (IW) (g); WG (%) = 100 × [(FW – IW)/IW]; SGR = 100 (Ln FW) – (Ln IW)]/experimental days); FCR = feed fed (g)/weight gain (g), and PER= weight gain (g)/protein fed (g). The measured biometric indices were the hepatosomatic (HSI) and viscerosomatic (VSI) indices and the condition factor (K). Hepatosomatic index (HSI) = 100× [liver weight (g)/ body weight (g)] and viscerosomatic index = 100 × [viscera weight (g)/body weight (g)]. Condition factor (CF) = 100 × [body weight (g)/ length^3 (cm)].

2.12. Fish biochemical analyses

The proximate composition of the whole fish body was estimated in triplicate samples. The contents of moisture, lipid, protein, and ash were all analyzed using the methodology of **AOAC (1995)**.

2.13. Blood samples

Immediately, at the end of the experiment, five fish per tank were randomly sampled for blood collection (15 fish per treatment), using non-heparinized syringes from the caudal vasculature, pooled for each tank and centrifuged at 3500xg for 10min. Serum samples were kept at –20˚C until analysis.

2.14. Routine blood analyses

Serum and blood analyses of uric acid, creatinine, glucose, alanine amino transferase (ALT), aspartate amino transferase (AST) and hemoglobin content (HB) were measured using commercial kits (Biotec, Egypt).

2.15. Preparation of tissue samples for oxidant and antioxidant biomarkers

The fish samples were weighed, decapitated, and dissected on ice. Liver and brain tissues were homogenized with nine volumes of potassium phosphate buffer, 0.1 M, pH 7.4, and then centrifuged at 3500*x*g for 15min, and the supernatant was kept at 4˚C to be subsequently used to measure the levels of thiobarbituric acid reactive substances (TBARS) **(Tappel & Zalkin, 1959**), nitric oxide (NO) **(Montgomery & Dymock, 1961)**, superoxide dismutase (SOD) and the reduced glutathione (GSH) **(Marklund & Marklund, 1974)**. The acetylcholinesterase (AChE) activity in brain tissue was measured following the method of **Jollow *et al*. (1974**).

2.16. Statistical analysis

All data were subjected to one-way ANOVA, followed by the Tukey's test to assess significant differences among groups at $P< 0.05$. All the statistical analyses were done using the SPSS program version 20 (SPSS, Richmond, VA, USA) as described in the study of **Dytham (2011)**.

Results

3. Current study results

3.1. Chemical compositions of samples

Table (2) compared the proximate compositions of FDDP to DDP throughout the time intervals of FDDP preparation, where day zero (Day 0) represents the start of inoculation or DDP; week one represents the 7th day after inoculation, and week two stands for day 14 after inoculation (FDDP formation). Dietary crude protein, fiber, organic matter and nitrogen free extract were higher in FDDP than in DDP.

Table 2. Proximate compositions (% dry weight) of FDDP and DDP

	Chemical composition (%)											
Time interval	**Moisture**	**Organic matter**	**Crude protein**	**Crude fiber**	**Ether extract**	**Nitrogen free extract**	**Ash**	**NDF**	**ADF**	**ADL**	**Hemicellulose**	**Cellulose**
Day 0	3.12±0.51^{b}	98.46±0.91^{b}	8.84±0.51^{b}	7.30±0.67^{b}	1.32±0.71^{b}	81.22±0.91^{a}	1.32±0.89^{b}	26.60±0.74^{b}	32.01±0.43^{a}	5.63±0.88^{b}	8.11±0.11^{c}	22.9±0.51^{a}
Week One	3.41±0.32^{a}	98.51±0.85^{b}	13.83±0.21^{a}	8.03±0.61^{a}	1.44±0.58^{a}	74.89±06^{1b}	1.94±0.14^{a}	27.80±0.32^{b}	31.10±0.11^{a}	6.61±0.59^{a}	11.50±0.38^{b}	21.9±0.1^{b}
Week Two	3.42±0.41^{a}	98.68±0.78^{a}	14.63±0.77^{a}	7.83±0.38^{a}	1.36±0.68^{b}	75.06±0.45^{b}	2.01±0.76^{a}	36.70±0.93^{a}	28.5±0.579^{b}	6.64±0.65^{a}	13.98±0.94^{a}	21.5±0.3^{b}

All values were expressed as Mean ± SE. Means with different letters represent significant difference $P<0.05$) within the same column, the mean with letter (a) was the highest value.

3.2. Mineral analysis of samples

Table (3) shows FDDP containing a two-fold positive increment of nitrogen, phosphorous, and potassium, compared to DDP. Moreover, FDDP had higher concentrations of calcium, magnesium, iron, and zinc, but lower concentrations of manganese and copper, compared to DDP (Day 0) at $P< 0.05$.

Table 3. Mineral analysis of FDDP and DDP

Time	N%	P%	K%	Ca (µg/g)	Mg (µg/g)	Fe (µg/g)	Zn (µg/g)	Mn (µg/g)	Cu (µg/g)	P (µg/g)
Day 0	1.32±0.21^{b}	0.28±0.01^{b}	0.22±0.02^{c}	34.11±1.47^{c}	12.70±1.21^{b}	2.04±0.82^{b}	3.90±0.38^{b}	0.18±0.08^{a}	0.06±0.02^{a}	1.13±0.68^{b}
Week one	2.54±0.33^{a}	0.46±0.02^{a}	0.33±0.01^{b}	53.04±1.51^{b}	21.97±1.32^{a}	3.49±0.37^{a}	5.38±0.72^{a}	0.10±0.04^{b}	0.05±0.01^{a}	1.36±0.31^{a}
Week two	2.57±0.12^{a}	0.52±0.04^{a}	0.41±0.01^{a}	57.98±0.85^{a}	22.17±1.41^{a}	3.44±0.73^{a}	5.48±0.24^{a}	0.11±0.02^{b}	0.08±0.02^{a}	1.27±0.21^{a}

All values were expressed as Mean ± SE. Means with different letters represent significant difference ($P< 0.05$) within the same column; the mean with letter (a) was the highest value.

3.3. Morphological characterization

Fig. (1a, b) shows transversal sections of date pit particles before and after fungi degradation. Fig. (1a) presents transversal sections of degraded date pit (DDP) particles before fungi degradation, revealing thick tubes with a solid surface. While Fig. (1b) displays transversal sections of date pits during fungi degradation (FDDP), where different stages of *Trichoderma reesei* fungi colonies, date pit microscopic tubes were separated from each other, and particle sizes started getting smaller and looser as the degradation process progressed.

3.4. Fungus enzymatic specific activities during FDDP preparation time course

Table (4) reveals the carboxy methyl cellulase and xylanase enzymes' specific activities throughout the experimental time course. Both enzymes' specific activities were significantly increased ($P<0.05$) by time till reaching their highest activities on the 14th day of inoculation, indicating that the degradation process was in progress, and FDDP was fully formed.

Table 4. Fungus enzymatic specific activities

Enzyme/Time interval	Carboxy methyl cellulase specific activity (IU/mg protein)	Xylanase enzyme specific activity (IU/mg protein)
Day zero (DDP)	1.17±0.01^{d}	0.45±0.02^{c}
Fifth Day	1.58±0.03^{c}	1.20±0.12^{b}
Week one	1.74±0.21^{b}	1.36±0.22^{a}
Week two	2.48±0.32^{a}	1.46±0.25^{a}

All values were expressed as Mean ± SE. Means with different letters represent significant difference ($P<0.05$) within the same column; the mean with letter (a) was the highest value.

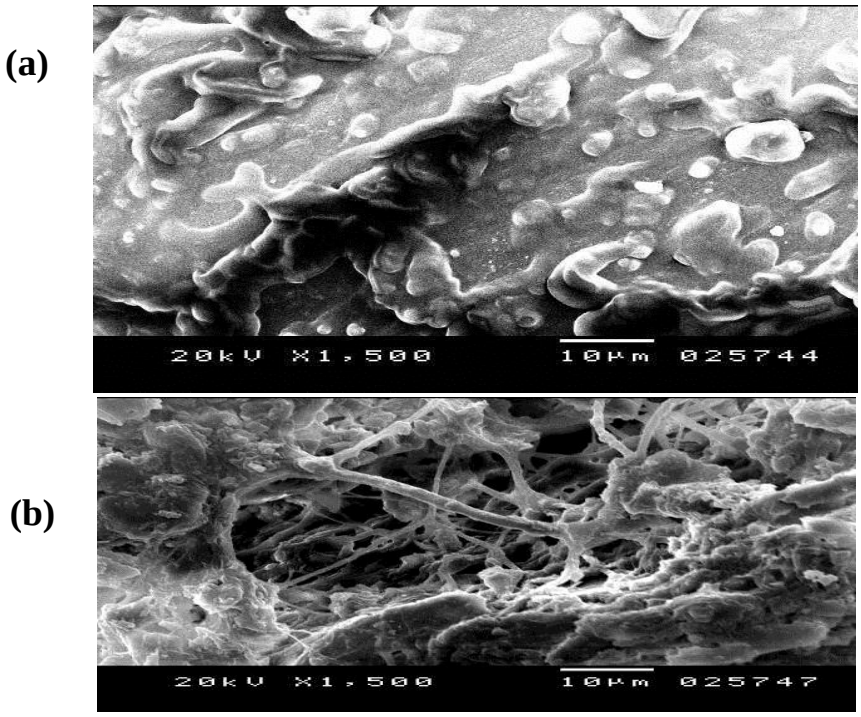


Fig. 1. Scanning electron micrograph of typical transversal section of date pits (**a**) Before degradation with *Trichoderma ressei* fungi and (**b**) During degradation with *Trichoderma ressei* fungi

3.5. Phytochemical compositions of DDP and FDDP extracts

As shown in Fig. (2), the highest values of total phenolics and flavonoids content were found in FDDP ethanolic extract (26.52 and 2.11mg/ 100g DW, respectively), followed by FDDP aqueous extract (25.67 and 1.97mg/ 100g DW, respectively).

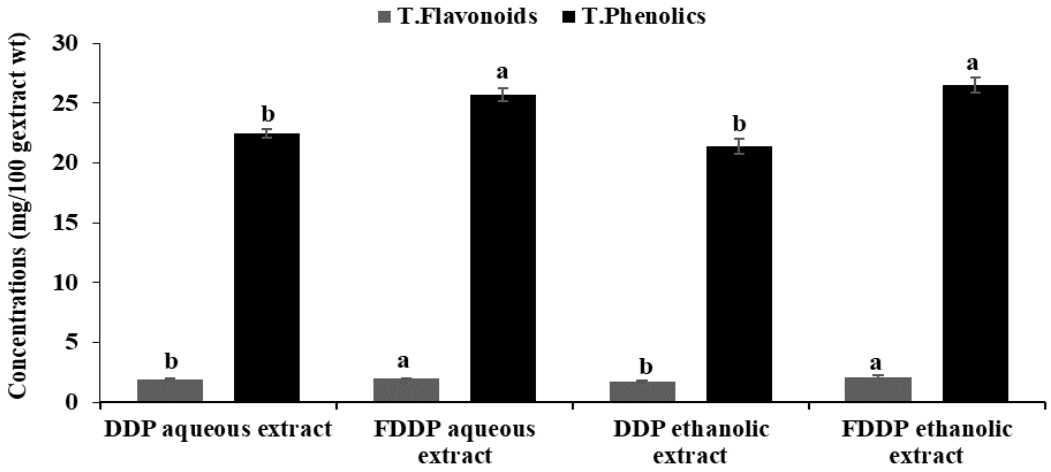


Fig. 2. Total flavonoids and total phenolics contents of DDP and FDDP ethanolic and aqueous extracts

3.6. The antioxidant activities of DDP and FDDP extracts

Table (4) shows the free radical scavenging activity using DPPH, and the nitric oxide scavenging activity of DPP and FDDP in aqueous and ethanolic extracts. Both DDP and FDDP extracts were radical scavengers. The radical scavenging activity of DDP "aqueous extract" increased from 15.89-51.28% as concentrations increased from 1–100 mg/mL, respectively; while, FDDP "aqueous extract" increased from 28.97– 59.69%. Scavenging activity increased significantly ($P< 0.05$) in the range of 16.29– 51.53%, respectively, in the case of DDP "ethanolic extract" and from 27.45–55.48% in the case of FDDP "ethanolic extract". However, their activities didn't exceed the antioxidant activity of the ascorbic acid (as a positive control), which showed the highest DPPH scavenging activity (87%) at a minimal concentration of 1mg/ ml. A reduction in nitric oxide radical formation indicates anti-inflammatory activity. Based on Table (5), both the FDDP extracts had higher anti-inflammatory activities compared to the DDP ones at all concentrations.

Table 5. The antioxidant and anti-inflammatory effect of DPP and FDDP extracts

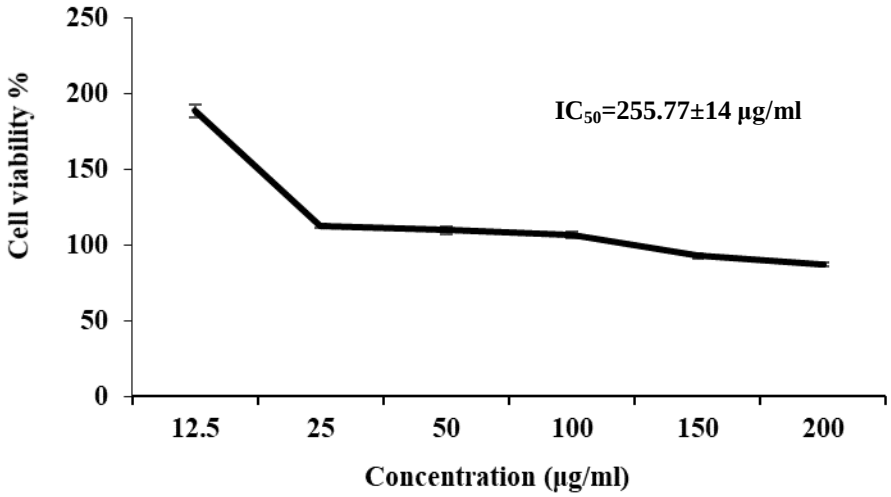
Concentrations (mg/mL)	%Radical scavenging activity				%NO scavenging activity			
	Aqueous extracts		Ethanolic extracts		Aqueous extracts		Ethanolic extracts	
	DDP	FDDP	DDP	FDDP	DDP	FDDP	DDP	FDDP
1	15.89±2.21^{c}	28.97±3.41^{d}	16.29±1.21^{c}	27.45±2.11^{b}	0.56±.0.11^{d}	25.76±2.26^{c}	0.45±.0.12^{d}	23.67±2.52^{c}
10	45.15±1.61^{b}	31.79±3.50^{c}	47.26±1.84^{b}	29.40±2.55^{b}	4.83±0.56^{c}	29.32±2.45^{b}	2.46±0.52^{c}	27.87±1.68^{b}
50	49.74±2.42^{a}	56.69±2.12^{b}	51.26±2.34^{a}	53.45±1.98^{a}	8.25±1.55^{b}	29.96±3.67^{b}	8.76±1.31^{b}	28.69±2.60^{b}
100	51.28^{a}±1.26^{a}	59.69±3.22^{a}	51.53±1.67^{a}	55.48±3.12^{a}	18.14±2.11^{a}	39.43±2.11^{a}	16.15±2.78^{a}	37.32±2.22^{a}
IC_{50} (mg/ml)	3.35±0.98	2.98 ±1.1	3.26±0.88	3.29 ±0.92	9.98 ±2.21	7.03±1.5	8.07±0.76	18.66±0.81
Ascorbic acid (1 mg/ml)	87.17				37.26			

All values were expressed as Mean ± SE. Means with different letters represent significant difference ($P< 0.05$) within the same column; the mean with letter (a) was the highest value.

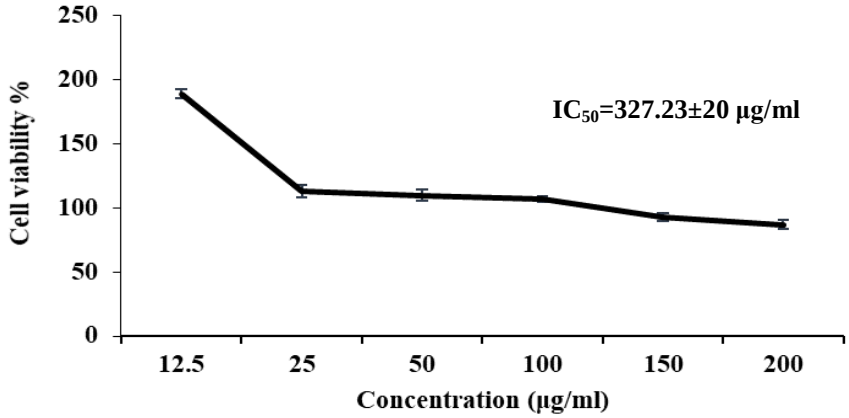
3.7. Cellular cytotoxicity assessment of DDP/FDDP extracts

Aqueous DDP extract (Fig. 3a), aqueous FDDP extract (Fig. 3b), ethanolic DDP extract (Fig. 3c) and ethanolic FDDP extract (Fig. 3d) had no cytotoxic effect on human WBCs up to 200g/ ml. FDDP ethanolic extract was the safest one because it exerted the highest IC_{50} value (614.58µg/ ml), followed by DDP ethanolic extract (368µg/ ml), and finally DDP aqueous extract (255.77µg/ ml) at $P< 0.05$.

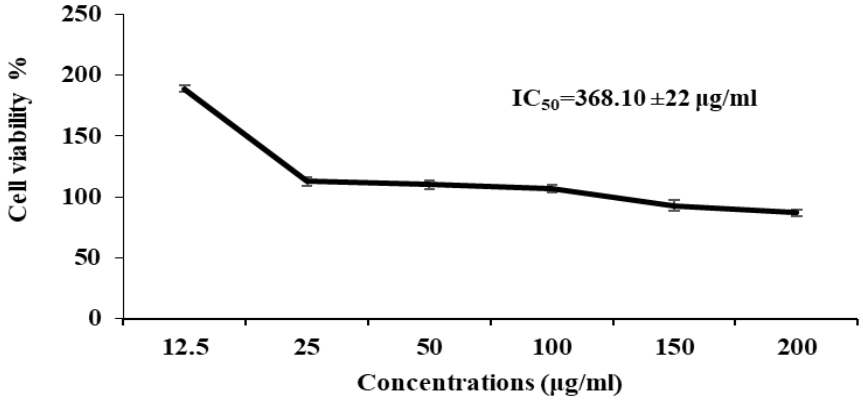
(a)


Cell viability %
250
200
150
100
50
0
IC50=255.77±14 µg/ml
12.5
25
50
100
150
200
Concentration (µg/ml)

(b)


Cell viability %
250
200
150
100
50
0
IC50=327.23±20 µg/ml
12.5
25
50
100
150
200
Concentration (µg/ml)

(c)


Cell viability %
250
200
150
100
50
0
IC50=368.10 ±22 µg/ml
12.5
25
50
100
150
200
Concentrations (µg/ml)

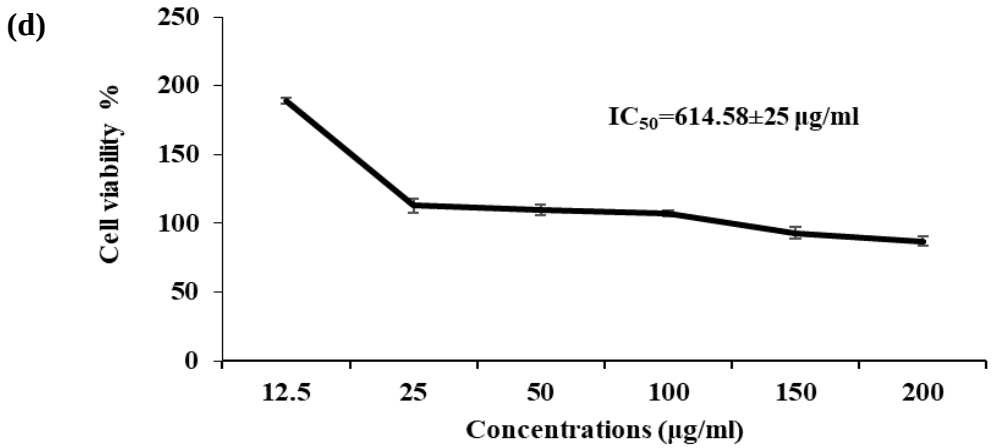


Fig. 3. Cell viability of WBCs treated with DDP and FDDP aqueous and ethanolic extracts after 72 hours

(**a**) WBCs treated with DDP aqueous extract, (**b**) WBCS treated with DDP ethanolic extract, (**c**) WBCs treated with FDPP aqueous extract, (**d**) WBCs treated with FDDP ethanolic extract.

3.8. HPLC polyphenols profile of DDP and FDDP ethanolic extracts

Results in Table (6) show the HPLC analysis of bioactive compounds extracted from DDP and FDDP 70% ethanolic extracts, where retention times and UV spectra were compared to standards (Supplementary Fig. 1). Chrysin, naringenin, luteolin, apigenin-7-O-Glucoside, sinapic acid, and protocatechuic acid were found at the highest concentrations in the FDDP ethanolic extract. Moderate concentrations were detected in gallic, caffeic, rosmarinic, cinnamic, kaempferol, and the lowest concentrations of cateachin, vanillic, and *p*-coumaric were recorded, compared to DDP ethanolic extract.

Table 6. HPLC identity of bioactive compounds present in DPP and FDDP ethanolic extracts

Compound	Sample/ concentration (µg/g)	
	DDP ethanolic extract	FDDP ethanolic extract
Gallic	11.734	32.196
Protocatechuic	75.125	210.152
p-hydroxybenzoic	2796.922	1363.961
Cateachin	53.414	11.482
Caffeic	10.021	17.938
Syringic	17.974	154.427
Vanillic	30.451	19.750
Ferulic	2.277	ND
Sinapic	292.374	467.276
p-coumaric	18.679	5.091
Apigenin-7-O- Glucoside	348.032	533.262
Rosmarinic	63.432	72.842
Cinnamic	8.443	10.762
Luteolin	62.781	95.285
Naringenin	ND	255.963
Apigenin	41.960	31.851
Kaempferol	5.148	7.310
Chrysin	1.927	37.384

3.9. Growth performance, feed utilization efficiency and biometric indices

Table (7) shows the results for the growth and feed utilization indices. Diets supplemented with D4 (1%) and D7 (2%) had the highest WG values (10.54% and 10.49%, respectively), except for D5 (0.5% crude FDDP; 0.94%) and D6 (1% crude FDDP; 0.950.15) diets, which showed a significant decrease in SGR levels when compared to the control group (D0) at $P< 0.05$. A non-significant difference in FCR level was detected at P 0.05 between different groups and the control one, except for D4 (1% ethanolic extract; 2.17%), D7 (2% crude FDDP; 2.12%) and D8 (2% crude FDDP; 2.25%) diets, which showed the lowest FCR levels ($P< 0.05$). All diets supplemented with FDDP ethanolic extract (D1-D4) and crude FDDP (D5-D8) showed non-significant differences in PER values, compared to the control group at $P< 0.05$, except for D5, which recorded the lowest significant ($P< 0.05$) PER value, compared to all the experimental groups.

At all inclusion levels, the use of FDDP, either in crude or ethanolic extract form, resulted in a significant increase ($P< 0.05$) in HSI values relative to the control group, with the exception of the D4 group, which showed insignificant variation with the control (D0) group ($P> 0.05$). Moreover, values of VSI showed a significant increase in all dietary fish groups, except for D4 and D7, which were insignificantly different from D0 ($P> 0.05$). Similarly, the condition factor (K) values significantly increased ($P< 0.05$) directly relative to the dietary supplementation levels of either FDDP crude or ethanolic extract, showing the highest condition factor value in the D4 and D7 experimental groups, and only the D1 group showed a significant difference, compared to the control (D0) group.

Table 7. Fish performance indices of *Oreochromis niloticus* at the end of the experimental feeding trial

Growth and feed utilization indices	Experimental diets								
	DO	D1	D2	D3	D4	D5	D6	D7	D8
IW	14.77±0.55	14.88±0.34	14.77±0.53	14.98±0.42	14.97±0.54	14.99±0.66	14.96±0.67	14.82±0.78	14.76±0.62
WG	9.99±0.19^{b}	9.12±1.38^{c}	9.58±1.18^{b}	9.64±1.23^{b}	10.54±0.16^{a}	9.12±0.98^{c}	9.41±0.45^{b}	10.49±0.26^{a}	10.42±0.84^{a}
SGR	1.15±0.22^{ab}	0.95±0.13^{bc}	1.02±0.10^{abc}	1.13±0.22^{ab}	1.19±0.16^{a}	0.94±0.08^{c}	0.95±0.15^{c}	1.18±0.12^{a}	1.08±0.17^{abc}
FI	24.06±0.7 5	23.24±0.96	23.06±0.92	23.31±0.72	23.06±0.65	23.55±0.10	23.44±0.84	22.41±0.52	23.47±0.48
FCR	2.39±0.21^{a}	2.40±0.38^{a}	2.41±0.20^{a}	2.26±0.90^{b}	2.17±0.33^{b}	2.38±0.25^{a}	2.36±0.10^{a}	2.12±0.13^{b}	2.25±0.23^{b}
PER	1.49±0.13^{ab}	1.19±0.15^{bc}	1.20±0.12^{bc}	1.30±0.39^{bc}	1.45±0.12^{ab}	1.07±0.14^{c}	1.20±0.27^{bc}	1.57±0.04^{b}	1.30±0.11^{bc}
HSI	1.78±0.02^{d}	2.79±0.07^{a}	2.71±0.35^{a}	2.19±0.09^{bc}	1.98±0.05^{cd}	2.72±0.01^{a}	2.63±0.15^{a}	2.33±0.25^{b}	2.30±0.07^{b}
VSI	7.19±0.59^{c}	9.93±0.94^{a}	8.97±0.48^{ab}	8.87±0.58^{ab}	7.88±0.18^{bc}	9.64±1.09^{a}	8.89±0.87^{a}	7.70±0.04^{bc}	8.14±0.33^{ab}
K	1.60±0.001^{c}	1.56±0.04^{c}	1.81±0.18^{b}	1.82±0.23^{b}	2.14±0.09^{a}	1.95±0.06^{ab}	1.94±0.02^{ab}	2.24±0.09^{a}	1.83±0.06^{b}

All values were expressed as Mean ± SE. Means with different letters represent significant difference ($P<0.05$) within the same column; the mean with letter (a) was the highest value.

D0, Control diet; D1-D4: Diets containing different concentrations of FDDP ethanolic extract (2.5, 5, 7.5, 10g/ kg diet), respectively; D5- D8: Diets containing different concentrations of crude FDDP (5, 10, 20, 30g/ kg diet), respectively. WG: Weight gain (g); FCR, Food conversion ratio; FI, Feed intake (g); HSI: Hepatosomatic index; IW: Initial body weight (g); K, Condition factor; PER, Protein efficiency ratio; SGR, Specific growth rate (%/day); VSI, Viscerosomatic index.

3.10. Fish biochemical composition

The carcass biochemical composition measured at the end of the experiment is presented in Table (8). The results showed that fish fed either D4 or D7 diets had significantly higher protein content than all other tested fish groups (P<0.05), with the exception of the control group (16.89%), where their protein contents were insignificantly different (P>0.05). On the other hand, results of fish biochemical composition indicate insignificant variation among all dietary groups (P>0.05) in the lipid content. An increase in fish moisture content was recorded upon feeding fish with FDDP either crude or ethanolic extract in comparison with the control group, except in the D4 and D7 groups where moisture contents (74.21 and 74.17%, respectively) were insignificantly different (P> 0.05) relative to the D0 group (74.65%). Except for the D8 diet group, which showed an insignificant difference with the control (D0) group, FDDP in both forms and inclusion levels increased the ash content relative to the control fish group (P< 0.05).

Table 8. Biochemical composition of *Oreochromis niloticus* at the end of the experimental feeding trial

Biochemical composition (%)	Experimental diets								
	DO	D1	D2	D3	D4	D5	D6	D7	D8
Crude protein	16.89±0.13^{ab}	16.11±0.66^{bc}	16.13±0.06^{bc}	16.18±0.22^{bc}	17.08±0.36^{a}	16.10±0.07^{bc}	16.23±0.42^{bc}	17.25±0.44^{a}	16.25±0.53^{bc}
Crude lipid	4.6±0.148^{a}	4.67±0.23^{a}	4.70±0.19^{a}	4.80±0.07^{a}	4.84±0.18^{a}	4.63±0.14^{a}	4.69±0.58^{a}	4.72±0.48^{a}	4.82±0.23^{a}
Moisture	74.65±0.08^{b}	75.26±1.26^{a}	75.18±0.18^{a}	75.09±0.37^{a}	74.21±0.68^{b}	75.22±0.14^{a}	75.01±0.87^{a}	74.17±0.73^{b}	75.27±0.90^{a}
Ash	3.25±0.08^{d}	3.48±0.11^{ab}	3.49±0.06^{a}	3.43±0.05^{ab}	3.34±0.05^{bc}	3.56±0.07^{a}	3.57±0.08^{a}	3.38±0.07^{b}	3.2±0.081^{cd}

All values were expressed as Mean ± SE. Means with different letters represent significant difference (P< 0.05) within the same column, the mean with letter (a) was the highest value.

D0, Control diet; D1-D4, Diets containing different concentrations of FDDP ethanolic extract (2.5, 5, 7.5, 10g/ kg diet), respectively; D5- D8: Diets containing different concentrations of crude FDDP (5, 10, 20, 30g/ kg diet, respectively).

3.11. Effect of FDDP supplementation on biochemical analyses of O. niloticus

Table (9) indicates that all experimental groups (D0-D8) had hyperglycemia except for D5, which had hypoglycemia and D4, which was normoglycemic (P< 0.05). All experimental groups showed significant increase in uric acid and creatinine levels at P< 0.05, compared to the control group but still in the non-chronic range of values, while the D4 and D7 groups showed a significant decrease at P< 0.05, compared to all groups. Identically, the ALT activity (U/L) of the groups of D1, D2, D3, D5, D6 and D8 was significantly higher than the control group (D0). The ALT activity (U/L) was lowest in groups D4 and D7 (p<0.05). Moreover, different experimental diets supplemented with variant concentrations of FDDP crude and ethanolic extract showed either the same or lower AST activities when compared to the D0 control group, especially the D4 and D7 groups, which had the lowest activities at p<0.05.

Table 9. Effect of FDDP supplementation on biochemical analyses of *O. niloticus*

Parameter	D0	D1	D2	D3	D4	D5	D6	D7	D8
Glucose (mg/dl)	61.90±1.21^{e}	67.45±2.35^{d}	156.85±1.56^{a}	98.71±2.73^{b}	62.78±2.26^{e}	31.47±1.81^{f}	93.95±2.52^{b}	85.95±1.89^{c}	73.42±1.46^{d}
HB (g/dl)	6.09± 0.13^{f}	6.11 ± 0.10^{f}	5.84± 0.18^{g}	6.77±0.01^{d}	9.80±0.51^{b}	7.11±0.27^{c}	6.30±0.02^{f}	10.18±0.07^{a}	6.54±0.13^{e}
Uric acid (mg/dl)	9.65±0.91^{d}	17.41±1.01^{b}	12.10±0.31^{e}	15.94±1.45^{b}	9.65±0.61^{d}	19.18±2.4^{4a}	13.74±1.31^{c}	9.66±0.31^{d}	18.59±0.53^{a}
Creatinine (mg/dl)	0.41± 0.04^{e}	0.54 ± 0.23^{d}	1.78± 0.21^{a}	1.30±0.41^{b}	0.23±0.04^{f}	0.85±0.27^{c}	1.22±0.16^{b}	0.24±0.06^{f}	0.49±0.03^{d}
ALT (U/L)	67.01±1.91^{d}	99.31±2.19^{a}	80.68±1.36^{c}	62.82±1.96^{c}	31.20±1.25^{g}	87.73±1.61^{b}	81.35±0.95^{c}	37.75±2.01^{f}	79.33±1.43^{c}
AST (U/L)	97.13± 1.73^{d}	148.08± 1.59^{a}	116.34±2.43^{b}	118.24±1.45^{b}	60.33± 1.03^{f}	101.82±1.26^{cd}	99.45±1.22^{d}	67.25±1.58^{e}	96.16±1.98^{d}

All values were expressed as Mean ± SE. Means with different letters represent significant difference (P<0.05) within the same column; the mean with letter (a) was the highest value.
D0, Control diet; D1-D4, Diets containing different concentrations of FDDP ethanolic extract (2.5, 5, 7.5, 10g/ kg diet, respectively); D5- D8 are diets containing different concentrations of crude FDDP (5, 10, 20, 30g/ kg diet, respectively).

3.12. The effect of FDDP supplementation on antioxidant capacity and associated oxidative stress in liver tissue

All experimental groups had higher liver GSH levels than the D0 control group (Fig. 4a). The D7 group showed the highest significant GSH level at P<0.05. Moreover, all groups had higher SOD activity than the control (D0), except D1, which had lower SOD activity. Among the diet groups supplemented with ethanolic extract (D1, D2 and D3), D4 had the highest significant SOD activity, while D7 had the highest significant SOD specific activity (Fig. 4b). The results indicate that, there was a significant reduction (P<0.05) in the liver TBARS levels of the D3, D4 groups, and D7 groups versus the D0 group. The D3 and D4 groups showed the lowest TBARS levels among other diet groups supplemented with FDDP ethanolic extract (D1, D2 and D3). The D7 group showed the lowest TBARS level among other diet groups supplemented with crude FDDP at P< 0.05 (Fig. 4c). Furthermore, all experimental groups showed a significant reduction in liver NO levels (P< 0.05) compared to D0, except for D2, which showed no difference with D0. While, the D1 group showed a significant increase in NO levels compared to all other groups; no differences were detected in NO levels between D4 and D7 among diet groups, supplemented with ethanolic extract or crude FDDP at P< 0.05 (Fig. 4c).

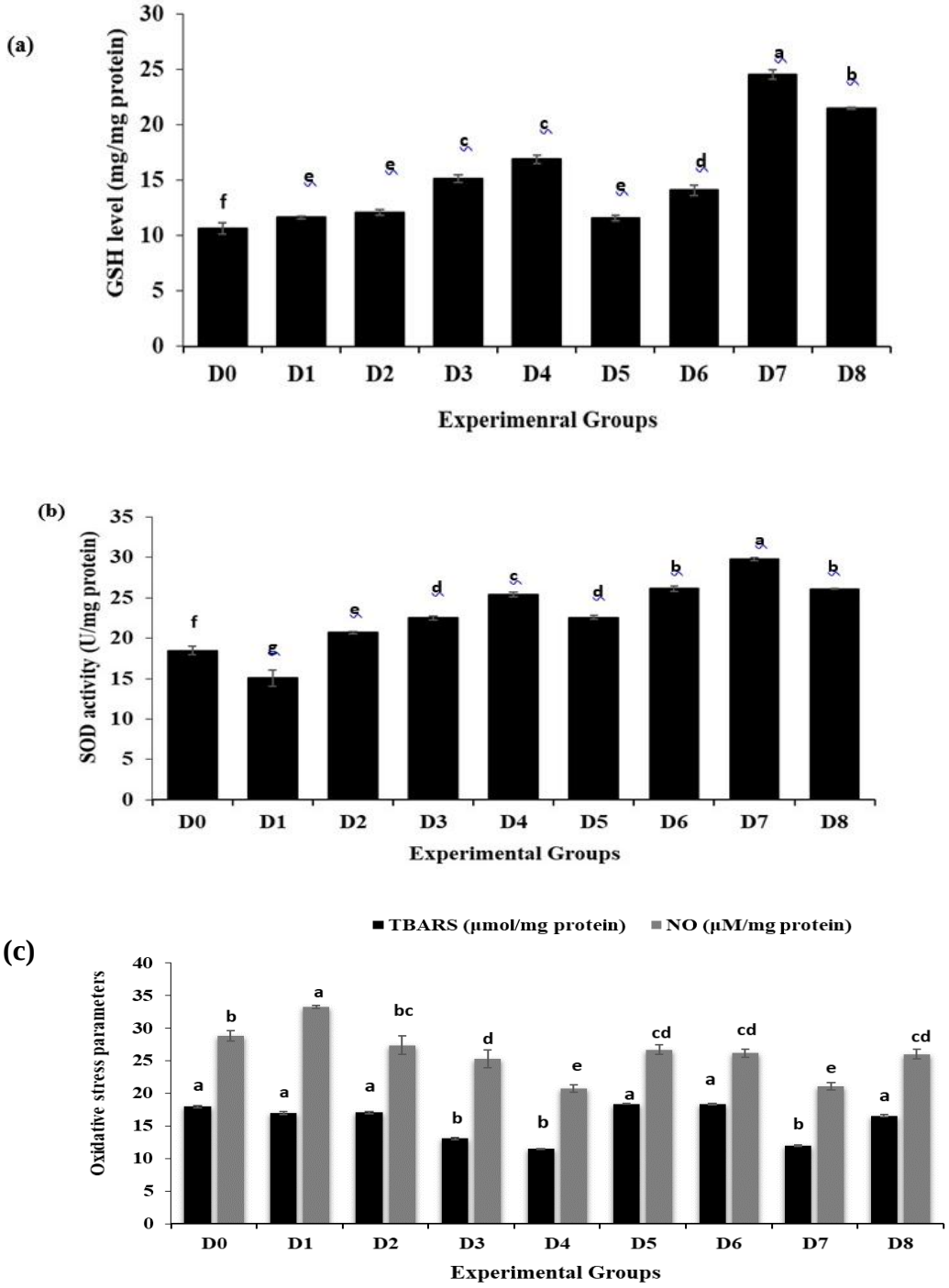
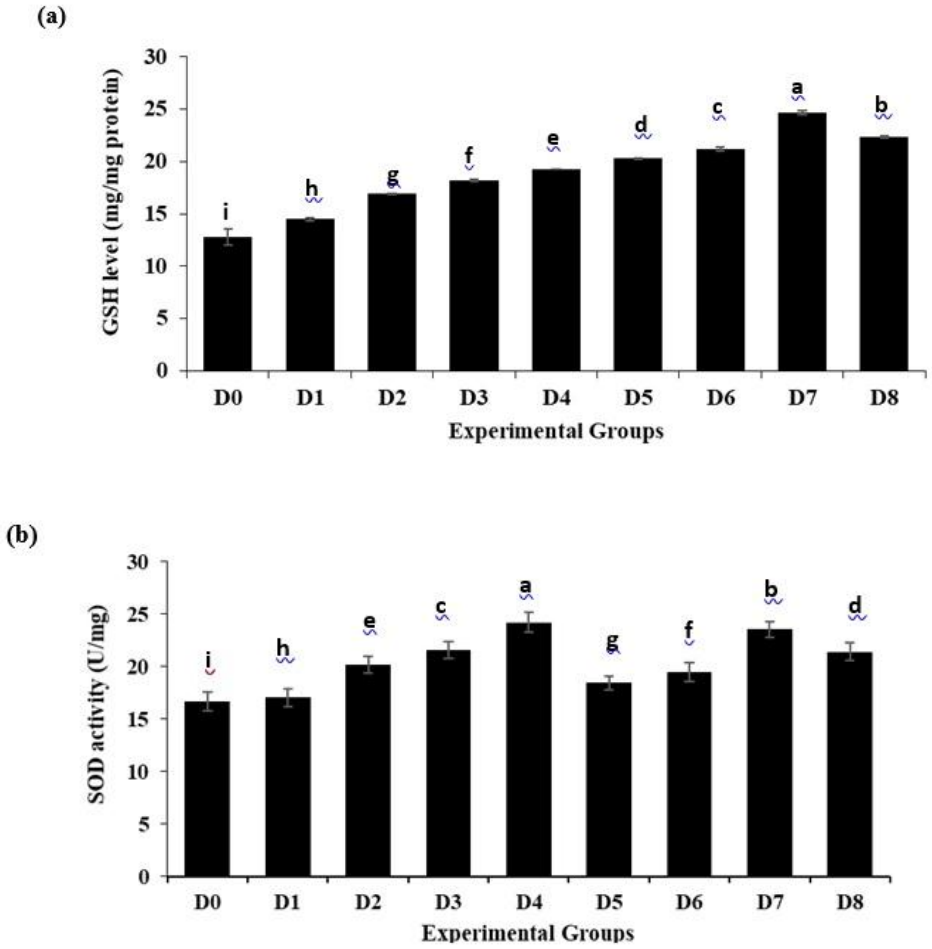


Fig. 4. Alterations in liver antioxidant capacity and associated oxidative stress parameters of different experimental groups

(**a**) liver GSH levels, (**b**) liver SOD activities, and (**c**) liver Oxidative stress biomarkers. All values were expressed as Mean ± SE. Means with different letters represent significant difference ($P< 0.05$) within the same column; the mean with letter (a) was the highest value. D0, Control diet; D1-D4 are diets containing different concentrations of FDDP ethanolic extract (2.5, 5, 7.5, 10g/ kg diet, respectively); D5- D8 are diets with different concentrations of crude FDDP (5, 10, 20, 30g/ kg diet, respectively).

3.13. The effect of FDDP supplementation on antioxidant capacity and associated oxidative stress in brain tissue

Results showed a significant increase (P<0.05) in brain GSH level and SOD specific activity (**Fig. 5a, b**) of all experimental groups, compared to D0 (control group). Among the diet groups supplemented with ethanolic extract, the D4 group had the highest GSH level and SOD specific activity. The D7 group had the highest GSH level and SOD specific among other diet groups supplemented with crude FDDP (D5, D6, D8) at P< 0.05. In all experimental groups (D2, D3, D4, D5, D6, D7, D8), the brain TBARS and NO levels were significantly reduced (**Fig. 5c**) compared to the D0 control group, except for the D1 group that showed a non-significant change. The D4 and D7 groups showed the lowest TBARS and NO levels among other diet groups supplemented with either FDDP crude or ethanolic extract. Additionally, the results indicate a significant decrease (P<0.05) in AChE specific activity in all experimental groups versus the D0 control group **(**Fig. 5d). Among the diet groups supplemented with either FDDP crude or ethanolic extract, the D4 and D7 groups had the lowest AChE specific activity.



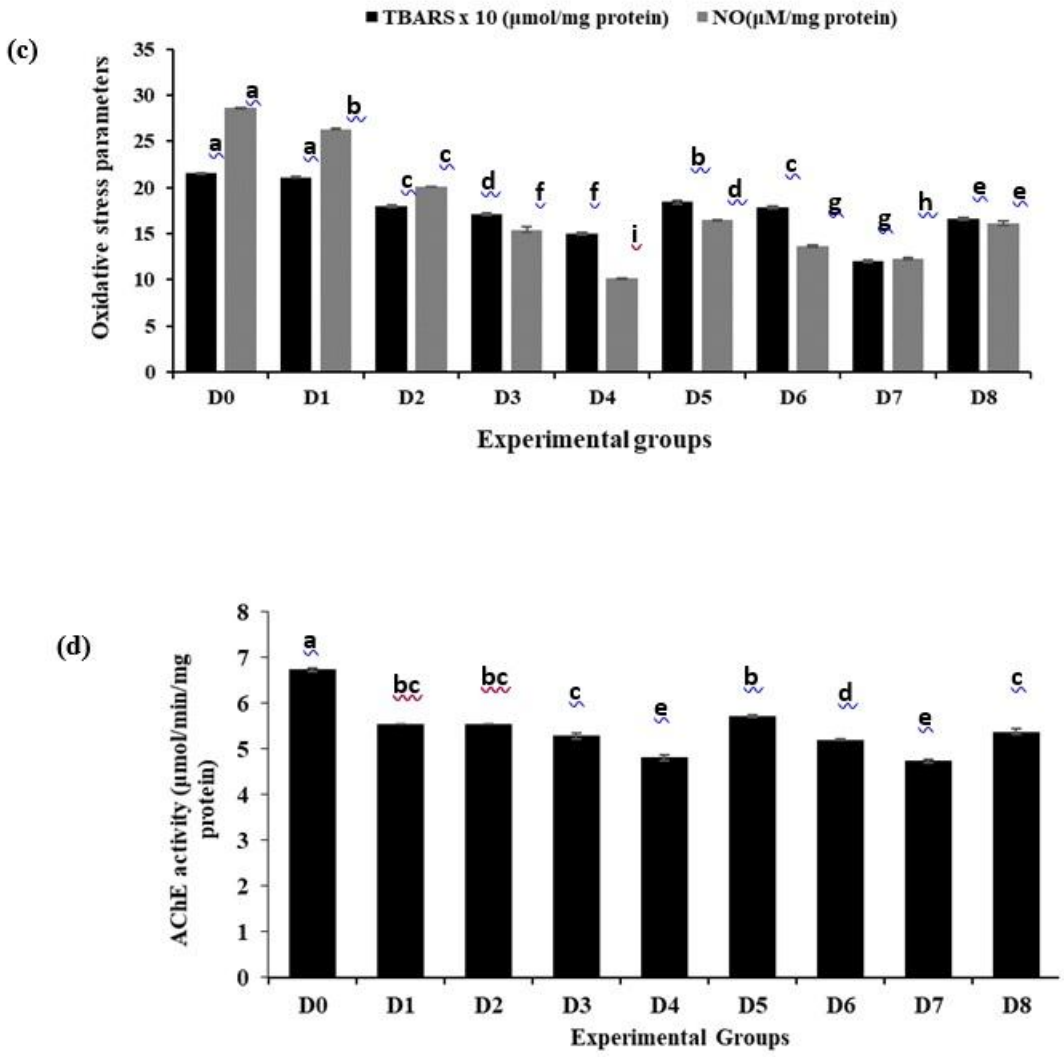


Fig. 5. Alterations in brain antioxidant capacity, associated oxidative stress parameters and AChE activity of different experimental groups

(**a**) brain GSH levels, (**b**) brain SOD activities, (**c**) brain Oxidative stress biomarkers, and (**d**) brain AChE activities. All values were expressed as Mean ± SE. Means with different letters represent significant difference ($P< 0.05$) within the same column; the mean with letter (a) was the highest value. D0, Control diet; D1-D4, Diets containeing different concentrations of FDDP ethanolic extract (2.5, 5, 7.5, 10g/ kg diet, respectively); D5- D8 are diets containing different concentrations of crude FDDP (5, 10, 20, 30g/ kg diet, respectively).

DISCUSSION

Date seed is a rich source of a wide variety of nutritive and bioactive compounds, including flavonoids, phenolics that are essential in reducing or controlling the harmful effects of oxidative stress. Besides, date seeds include nutritive compounds such as essential oils, vitamins and minerals (**Al-Zubaidy *et al.*, 2016; Theyab *et***

***al*., 2017)**. Owing to its high content of phytonutrients with antioxidant properties, date seeds have some health advantages, viz. reducing lipid oxidation, oxidative stress and free radical damage. Date pits have more protein, fiber and fat than date pulp. Date pits are used to feed fish in the Arabian Gulf **(Belal & Al-Owafeir, 2005)** in addition to their use in feeding sheep and cattle **(Al-Farsi & Lee, 2011)**. Carbohydrates found in date pits must be broken down by specific enzymes before used by animals, particularly by fish **(Belal, 2008)**. Hydrolysis of cellulose and hemicellulose by xylanases enzymes releasing nutrients bound to these fibers commonly occur in nature (**Leisola *et al*. (2002**). Viscosity is reduced and feed utilization is improved, resulting in less waste. Since enzymes in animal feed are expensive, using microorganisms that produce xylanases is a more cost-effective option for degrading dietary fiber. Plant's macronutrients are broken down into organic acid, simple sugars and free amino acids **(Nkhata *et al*., 2018; Xiang *et al*., 2019)**. In the current study, *Trichoderma reesei* was used, which is the most studied cellulolytic fungus **(Foreman *et al*. (2003)** to prepare FDDP. *Trichoderma reesei* degradation process was demonstrated in the present study both physically and chemically.

The electron microscope picture of freeze-dried date pits before degradation confirmed tightly packed structures and low nanosized- pore volume in accordance with a previous study **(Hossain *et al*., 2014)**. While, the different *Trichoderma reesi* colonies present indicate that *Trichoderma reesei* grew on date pits material by reducing stickiness and breaking down date pits particles. This raise in date pits particles surface areas that are exposed to digestive enzymes in the fish gut as mentioned by **Belal (2008)**.

In our study, after two weeks of inoculation process and in agreement with **Belal (2008)**, FDDP crude protein, crude fiber, ash, moisture, NDF and hemicellulose degradation increased, while ADF and cellulose decreased compared to DDP. It indicates chemical changes during degradation; decrease in total carbohydrate, increase in free sugars and digestible carbohydrate content were also observed. These results surpass all previous studies on date pits (**Bouhlali *et al*., 2016; El-Rahman & Al-Mulhem, 2017; Metoui *et al*., 2019)**. This may be due to the liberation of nutrients blocked within date pits fibers that would have led to more digestible nutrients from the DDP. **Leisola *et al*. (2002)** postulated that, xylanases (produced by *Trichoderma reesei*) liberate nutrients either by hydrolysis of non-degradable cellulose or hemicelluloses. Two weeks after inoculation, xylanase and carboxymethyl cellulase activities increased twice, confirming the degradation process in agreement with **Zhang *et al*. (2012)**.

According to **Rahman *et al*. (2007)**, date pits contain varying amounts of macro and trace elements depending on date cultivar, climate, irrigation water, and

fertilizers used to harvest them. Fortification of food products with FDDP is possible due to the element and mineral contents. **Adeosun and Bouhlali (2016)** reported higher potassium, phosphorous, magnesium, and zinc content than reported in our study.

Since polyphenols solubility varies depending on solvent polarity **(Maqsood *et al*., 2020)**. In the present study, we prepared aqueous and 70% ethanolic extracts of DDP and FDDP. The ethanolic extract had the highest phenolic and flavonoid contents. Our results were higher than those of **Hussain *et al*. (2020)** who studied total phenolic contents in six varieties of date palm pits extracted in different organic solvents.

The extract IC_{50} is inversely associated with its richness in antioxidant compounds, in other words, lower IC_{50} value indicates a higher antioxidant activity **(Al-Farsi & Lee, 2008)**. Our results exhibited higher scavenging activity of FDDP and DDP than values recorded in previous studies **(Al-Farsi & lee, 2008; Thouri *et al*., 2017)**. Interestingly, our findings revealed that, the FDDP aqueous extract has the highest NO scavenging activity, followed by FDDP ethanolic extract. The lower IC_{50} of our extracts indicates greater antioxidant activity. Date seeds are able to quench NO, thus they are very important in avoiding the negative effects of excessive NO production **(Bouhlali *et al*., 2020)**.

The highest IC_{50} value was obtained for FDDP ethanolic extract and it corresponded to be the safest, compared to other extracts, while DDP aqueous extract had the lowest IC_{50} value, making it the most toxic to human WBCs. Similarly, **Dawood *et* al. (2020)** found that, adding fermented date seed meal to the diet of the Nile tilapia improved their hematological parameters (RBCs, WBCs and HB). This explains their immunostimulatory and anti-infection properties (**Apeh *et al*., 2014)**.

The HPLC chromatogram of polyphenolic content of DDP and FDDP ethanolic extracts revealed that, the extracts displayed phenolic profiles with great differences, with the highest concentrations of hydroxy benzoic acid (p-hydroxy benzoic acid) and syringic acid and two cinnamic acid derivatives (caffeic and sinapic acids) that were found in FDDP ethanolic extract. Moreover, apigenin-7-O-Glucoside, luteolin, naringenin and chrysin were found in higher concentration in FDDP ethanolic extract, compared to DDP. In accordance with **Ahmed (2017)** and **Eid *et al*. (2014)**, the most abundant phenolic compounds in (ajwa) date seeds are p-coumaric acid, ferulic acid, gallic acid, caffeic acid, *p*-hydroxybenzoic acid, and syringic acid, with luteolin and apigenin forming the most abundant flavonoids.

The beneficial effects of using dates as a feed additive on fish performance including the Nile tilapia **(Belal, 2008; Azaza *et al*., 2009; Gaber *et al*., 2014)**, the African cat fish **(Sotolu *et al*., 2014)** and common carp **(Hoseinifar *et al*., 2017)** were widely documented. However, a few studies such as **Belal and Al-Owafeir (2005)** and **El-Sayed *et al*. (2006)** found that, date seed supplementation reduced tilapia growth and body fat. This may be due to the presence of high levels of crude fiber and non-digestible carbohydrates in date pits. Contrarily, **Belal (2008)** observed that, the weight gain was increased after the administration of FDDP as a feed supplement at concentrations of 150, 300, 450 & 600mg/ kg. This indicates that FDDP affected positively fish growth and attributed the increment in fish growth to the increase of digestible carbohydrates contents and the liberated nutrients and also to the high levels of free sugar mannose as a result of *Trichoderma reesei* fungi action.

Remarkably, in our study we found that, diets supplemented with D4 (1% ethanolic FDDP extract) and D7 (2% Crude FDDP) groups grew faster than all other tested groups. In addition, they significantly increased PER and SGR values. The improvement in nutrient utilization may be attributed to the increment of polyphenolic compounds level in FDDP, which act as natural antioxidants. Furthermore, fermentation process induces degrading of proteins into smaller peptides, which are more efficiently absorbed by intestinal cells **(Dawood *et al*., 2020)**.

We noticed the increase in HSI, VSI, and K values at D4 and D7 diets in accordance with **Kamali-Sanzighi *et al*. (2019)** who found that, adding 10% date seeds powder to a common carp diet increases growth but adding more date pits may reduce fish growth due to high fiber content and unsaturated fatty acids such as oleic acid **(Sotolu *et al*., 2014)**. In addition, adding FDDP in both forms significantly raises K values, compared to the control group, indicating improved fish welfare in accordance with the findings of **Hossain *et al*. (2014)** who attributed this improvement to the presence of beneficial bioactive materials in date pits.

After 10 weeks of feeding, our results revealed the dietary differences in fish biochemical compositions, where fish fed on FDDP, either in crude or extract forms, showed higher protein content, indicating the improvement in feed utilization through altering protein synthesis and muscle deposition rate **(Abdel-Tawwab *et al*., 2008)**. The anti-bacterial activity of FDDP extract or crude reduces pathogens in fish intestine, allowing more nutrients to be absorbed and converted into body mass **(Assem *et al*., 2014)**.

Furthermore, fish welfare was confirmed through biochemical analyses, where the highest hemoglobin content was found in D4 and D7 groups, indicating increased oxygen supply and consequently increased health effects on fish **(Vahedi *et al*., 2017)**. Although the D4 group showed normoglycemia, the addition of FDDP crude or ethanolic extract to fish feed diets resulted in significant increase in blood sugar concentration, which concurs with the findings of both **Masoudi *et al.* (2011)** and **Kamali-Sanzighi *et al.* (2019)** who stated that, glucose was highly accentuated when fibrous diets were added to the food in common carp and broilers, respectively, as fibrous material helped to increase the activity of gluconeogenesis.

Further, the presence of polyphenols as naringenin, chrysin and luteolin exhibits potent anti-hyperuricemic and anti-inflammatory effects **(Uckun *et al*., 2020)**, which made FDDP supplementation in fish diets beneficial. Date seeds also protect against hepatotoxicity and renal toxicity by detoxifying free radicals and inhaling lipid peroxidation and nitric oxides **(Mousalamy & Hussein, 2016; Ahmed, 2017)**.

Liver enzymes are important indicators of liver function where any increase in these enzymes may indicate liver damage **(Wang *et al*., 2001)**. In agreement with previous study, adding FDDP ethanolic extract (at concentration of 10g/ kg diet (D4 group) and FDDP crude (at concentration of 20g/ kg diet (D7 group) to fish feed diets for 45 days reduced liver enzyme activity **(Kamali-Sanzighi *et al*., 2019)**. Date pits' hepatoprotective effect was linked to polyphenolic compounds and minerals like zinc, copper, potassium and manganese **(Saryono & Proverawati, 2019)**.

Notably, supplementing fish diets with FDDP increased GSH levels in both liver and brain homogenates, especially at D4 and D7 concentrations. Antioxidant enzyme as SOD act as a defense mechanism against ROS as SOD decomposes superoxide radical (O_2^-) to produce H_2O_2. SOD activity in liver and brain increased by increasing of concentration of FDDP supplementation either crude or ethanolic extract in fish diet especially in D4 and D7 diets. Besides, FDDP's ability to reduce accumulation of free radicals caused by lipid peroxidation or stresses on fish was explained. Similarly, **Alyileili *et al*. (2020)** found that supplementing broiler feed with 10% FDDP increased antioxidant enzyme syntheses such as SOD, GPX.

In our study, FDDP crude and ethanolic extracts reduced TBARS and NO levels in liver and brain homogenates, with the lowest values at D4 and D7 diets indicating that FDDP inhibits lipid peroxidation and NO production by inhibiting NOS activity. Likewise, **Maqsood *et al*. (2020)** and **Hoseinifar *et al*. (2017)**

stated that date seeds extracts possess protective and anti-inflammatory effects. This is related to their activity in enhancing activities of antioxidants as SOD, GST, and reduction in TBARS level, thus thy acted as good antioxidants.

In agreement with **Sumanth and Mamatha (2014)** who found that *Phoenix dactylifera* enhanced learning and memory in the Morris water maze and suggested its mechanism by decreasing acetylcholine esterase activity leading to accumulation of acetylcholine and thus enhancing memory. In our work, we found that either supplemented crude or ethanolic extract of FDDP especially at concentration of 1% ethanolic extract (D4) and 2% crude extract (D7) decreased AChE specific activity. In fact, dates are rich in polyphenolic compounds that have anticholinesterase activities, so FDDP can act as cholinergic system activator or free radical scavenger (**Tumiatti *et al*. (2008**).

We had screened various concentrations of both FDDP crude and ethanolic extract to identify which ones were most comparable to the control group and had the most effective biological and physiological impacts on fish health. Our results indicated that they were (D4 and D7) groups in FDDP ethanolic and crude; respectively. We intended to use these concentrations in our subsequent researches as the most potent FDDP supplements for fish diets.

CONCLUSION

The present study revealed that utilization of fungi degraded date pits as a feed additive is capable of improving fish growth performance and nutrient utilization as well as ensuring production of healthy fish on sustainable basis. Supplementation of 1% ethanolic FDDP extract and/or 2% crude FDDP in tilapia diet enhanced the growth performances by improving final weight, feeding efficiency and specific growth rate. This study further revealed that FDDP incorporated diet did not show any adverse effect in body composition and hematological profile in the tilapia but also enhanced oxidant and antioxidant status. We recommend the use of FDDP as an excellent feed additive in Nile tilapia diets.

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REFERENCES

Abdel-Tawwab, M.; Abdel-Rahman, A.M. and Ismael, N.E.M. (2008). Evaluation of commercial live bakers' yeast, Saccharomyces cerevisiae as a growth

and immunity promoter for Fry Nile tilapia, Oreochromis niloticus (L.) challenged in situ with Aeromonas hydrophila. Aquaculture 280:185-189.

Abo-State, H.; El-Monairy, M.; Hammouda, Y. and Elgendy, M. (2017). Effect of a Phytogenic Feed Additive on the Growth Performance and Susceptibility of Oreochromis niloticus to Aeromonas hydrophila. J Fish Aquat Sci 12: 141-148.

Ahmed, I. (2017). Effects of dietary amino acid l-lysine on survival, growth and haemato-biochemical parameters in Indian catfish, Heteropneustes fossilis (Bloch, 1974), fingerlings. J. Appl. Ichthyol 33:1027-1033.

Aldosari, M.; Al–Hozab, A.; Hmeidan, M. and Alothaymeen, I. (1995). Discarded dates and wheat straw as ingredients in the rations of growing sheep. Arab Gulf J. Sci. Res 13:123-131.

Al-Farsi, M.A. and Lee, C.Y.(2008). Optimization of phenolics and dietary fibre extraction from date seeds. Food Chem 108:977-985.

Al-Farsi, M.A. and Lee, C.Y. (2011). Usage of Date (Phoenix dactylifera L.) Seeds in Human Health and Animal Feed. In Nuts and Seeds in Health and Disease Prevention; Preedy, V.R., Watson, R.R., Patel, V.B., Eds.; Elsevier: Amsterdam, The Netherlands, 2011; Chapter 53, pp. 447–452.

Alyileili, S.R.; El-Tarabily, K.; Ibrahim, W.H.; Sulaiman, M. and Hussein, A.S. (2020). Effect of Trichoderma reesei Degraded Date Pits Supplementation on Growth Performance, Immunoglobulin Levels, and Intestinal Barrier Functions of Broiler Chickens. Recent Pat. food, Nutr. Agric 11:168-181.

Al-Zubaidy, N.; Al-Zubaidy, A. and Sahib, H. (2016). The anti-proliferative activity of phoenix dactylifera seed extract on MCF-7 breast cancer cell line. Int. J. Pharm. Sci 41:358-362.

AOAC. (1990). Official Methods of Analysis, Association of Official Analytical Chemists. 15th ed. Washington, USA.

AOAC. (1995). Official methods of analysis of AOAC International. 6th ed. Association of Official Analytical Chemists, Arlington, VA, USA pp 1141.

Apeh, V.; Agu, C.; Ogugua, V.; Uzoegwu, P.; Emeka, A.; Rex, T. and Agbalu, I. (2014). Effect of Cooking on Proximate, Phytochemical Constituents and Hematological Parameters of Tetracarpidium conophorum in Male Albino Rats. European J Med Plants 4:1388-1399.

APHA, A.WEF. (1995). Standard methods for the examination of water and wastewater (19th ed.). American Public Health Association.

Assem, H.; Khalifa, A. and Elsalhia, M. (2014). Physiological and microbiological indices as indicators of evaluating dietary fungi degraded date pits as a probiotic for cultured Nile tilapia Oreochromis niloticus fingerling and its effect on fish welfare. Egypt. J. Aquat. Res 40: 435-441.

Association of Official Analytical Chemists [AOAC]. (1990). Official methods of analysis of AOAC international (15th ed.). AOAC.

Attia, A.I.; Reda, F.M.; Patra, A.K.; Elnesr, S.S.; Attia, Y.A and Alagawany, M. (2021). Date (Phoenix dactylifera L.) by-Products: Chemical Composition, Nutritive Value and Applications in Poultry Nutrition, an Updating Review. Animals 11:1133.

Azaza, M.S.; Mensi, F.; Kammoun, W.; Abdelouaheb, A.; Brini, B. and KraÏEm, M. (2009). Nutritional evaluation of waste date fruit as partial substitute for soybean meal in practical diets of juvenile Nile tilapia, Oreochromis niloticus L. Aquac. Nutr 15: 262-272.

Belal, I.E.H. (2008). Evaluating fungi-degraded date pits as a feed ingredient for Nile tilapia Oreochromis niloticus L. Aquac. Nutr 14:445-452.

Belal, I.E. and Al- Owafeir, M. (2005). Incorporating date pits Phoenix dactylifera and their sprouts in semi- purified diets for Nile tilapia Oreochromis niloticus (L.). J World Aquac Soc 35: 452-459.

Bharathi, S.; Antony, C.; Rajagopalasamy, C.; Arumugam, U.; Ahilan, B. and Aanand, S. (2019). Functional feed additives used in fish feeds. Int. J. Fish. Aquat. Stud 7: 44-52.

Bouhlali, E.D.T.; Alem, C. and Zegzouti, Y.F. (2016). Antioxidant and anti-hemolytic activities of phenolic constituents of six moroccan date fruit (Phoenix dactylifera L.) syrups. J. glob. innov. agric. soc. Sci 12: 45-52.

Bouhlali, E.d.T.; Hmidani, A.; Bourkhis, B.; Khouya, T.; Ramchoun, M.; Filali-Zegzouti, Y. and Alem, C. (2020). Phenolic profile and anti-inflammatory activity of four Moroccan date (Phoenix dactylifera L.) seed varieties. Heliyon. 6, e03436.

Chang, C.C.; Yang, M.H.; Wen, H.M. and Chern, J.C. (2002). Estimation of Total Flavonoid Content in Propolis by Two Complementary Colorimetric Methods. J Food Drug Anal 10:178-182.

Dawood, M.A.O.; Eweedah, N.M.; Khalafalla, M.M. and Khalid, A. (2020). Evaluation of fermented date palm seed meal with Aspergillus oryzae on the

growth, digestion capacity and immune response of Nile tilapia (Oreochromis niloticus). Aquac. Nutr. 26, 828-841.

Dytham, C. (2011). Choosing and Using Statistics: A biologist's Guide. Blackwell Science Ltd, London.

Eid, N.; Enani, S.; Walton, G.; Corona, G.; Costabile, A.; Gibson, G.;Rowland, I. and Spencer, J.P. (2014). The impact of date palm fruits and their component polyphenols, on gut microbial ecology, bacterial metabolites and colon cancer cell proliferation. J. Nutr. Sci 3:e46.

Elgasim, E.A.; Alyousef, Y.A. and Humeid, A.M. (1995). Possible hormonal activity of date pits and flesh fed to meat animals. Food Chem. 52:149-152.

El-Rahman, S. and Al-Mulhem, S. (2017). Characteristic analysis, antioxidant components and antioxidant activity of date fruits, DateSeeds and palm shell. Clin. Med. Case Rep 1:1-5.

El-Sayed, A.F.M.; Hamza, W.R. and Al-Darmaki, M. (2006). Evaluation of date pits as a feed ingredient for juvenile and adult Nile tilapia (Oreochromis niloticus) reared in a recirculating system. Paper presented at the Proceeding of 7th International Symposium on Tilapia aquaculture, Mexico.

Fang, H.; Li, C.; Zhao, J. and Zhao, C. (2021). Biotechnological Advances and Trends in Engineering Trichoderma reesei towards Cellulase Hyperproducer. Biotechnol.Bioprocess Eng 26:517–528.

Food and Agriculture Organization [FAO]. (2011). The state of world fisheries and aquaculture 2011.

Food and Drug Administration [FDA]. (1993). Subchapter E - Animal drugs, feeds, and related products.

Foreman, P.K.; Brown, D.; Dankmeyer, L.; Dean, R.; Diener, S.; Dunn-Coleman, N.S.; Goedegebuur, F.; Houfek, T.D.; England, G.J.; Kelley, A.S.; Meerman, H.J.; Mitchell, T.; Mitchinson, C.; Olivares, H.A.; Teunissen, P.J.; Yao, J. and Ward, M.(2003). Transcriptional regulation of biomass-degrading enzymes in the filamentous fungus Trichoderma reesei. J. Biol. Chem 278:31988-31997.

Gaber, M.; Basha, S.; Shatby, E.; Basha, S.; Shatby, E.; Basha, S.; Shatby, E.; Basha, S.; Shatby, E. and Basha, S. (2014). Effect of partially replacing corn meal by wet date on growth performance in Nile Tilapia (Oreochromis niloticus) fingerlings, diets supplemented with Digestarom®. GEP. 2, 60-67.

Garratt, D.C. (1964). The quantitative analysis of drugs (3rd ed.). Chapman Hall Ltd.

Gerber, F.; Krummen, M.; Potgeter, H.; Roth, A.; Siffrin, C. and Spoendlin, C. (2004). Practical aspects of fast reversed-phase high-performance liquid chromatography using 3μm particle packed columns and monolithic columns in pharmaceutical development and production working under current good manufacturing practice. J. Chromatogr.A 1036:127-133.

Guizani, N.; Suresh, S. and Rahman, M.S. (2014). Polyphenol contents and thermal characteristics of freeze-dried date pits powder. Proceedings of the International Conference of Agricultural Engineering, Zurich, Ref: C0296:1- 8.

Hass, S.; Bauer, JL.; Adakli, A.; Meyer, S.; Lippemeier, S.; Schwarz, S. and Schulz, C. (2016). Marine microalgae Pavlova viridis and Nannochloropsis sp. as n-3 PUFA source in diets for juvenile European sea bass (Dicentrarchus labrax L.). J Appl Phycol 28:1011–1021.

Hoseinifar, S.H.; Dadar, M.; Khalili, M.; Cerezuela, R. and Esteban, M.Á. (2017). Effect of dietary supplementation of palm fruit extracts on the transcriptomes of growth, antioxidant enzyme and immune-related genes in common carp (Cyprinus carpio) fingerlings. Aquac. Res 48:3684-3692.

Hossain, M.; Waly, M.; Singh, V.; Sequeira, V. and Rahman, M. (2014). Chemical Composition of Date-Pits and Its Potential for Developing Value-Added Product -a Review. Polish J. Food Nutr. Sci 64:215-226.

Hussain, M.I.; Farooq, M. and Syed, Q.A. (2020). Nutritional and biological characteristics of the date palm fruit (Phoenix dactylifera L.)–A review. Food Biosci 34:100509.

Jollow, D.J.; Mitchell, J.R.; Zampaglione, N. and Gillette, J.R. (1974). Bromobenzene-induced liver necrosis. Protective role of glutathione and evidence for 3,4-bromobenzene oxide as the hepatotoxic metabolite. Pharmacology 11:151-169.

Kalantar-zadeh, K. and Fry, B. (2008). Characterization Techniques for Nanomaterials. In: Nanotechnology-Enabled Sensors (eds. K. Kalantar-zadeh & B. Fry:211-281. Cham: Springer.

Kamali-Sanzighi, M.; Akrami, R.; Ghelichi, A. and Shamloofar, M. (2019). Partial replacement of plant sources by waste date (Phoneix dactylifera) in the diet of fingerling common carp (Cyprinus carpio) on growth performance, feed

utilization, hematological parameters and resistance to stress. Turkish J. Fish. Aquat. Sci 19:775-784.

King, M. and Vessal, M. (1969). "Enzymes of the Cellulase Complex," Advances in Chemistry, Vol. 95, 1969, pp. 130149.

Leisola, M.; Jokela, J.; Pastinen, O.; Turunen, O. and Schoemaker, H. (2002). Industrial use of enzymes. In S. a. C. O. U. United Nations Educational (Ed.), Encyclopedia of life support systems (EOLSS) (p.p. 1-9). EOLSS Publishers Co.

Maqsood, S.; Adiamo, O.; Ahmad, M. and Mudgil, P. (2020). Bioactive compounds from date fruit and seed as potential nutraceutical and functional food ingredients.
Food Chem 308:125522.

Marklund, S. and Marklund, G. (1974). Involvement of the superoxide anion radical in the autoxidation of pyrogallol and a convenient assay for superoxide dismutase. Eur J Biochem 47:469-474.

Masoudi, A.; Chaji, M.; and Mirzadeh, K. (2011). Effects of different levels of date pits on performance, carcass characteristics and blood parameters of broiler chickens. J.Appl. Anim. Res 39:1-7.

Metoui, M.; Essid, A.; Bouzoumita, A. and Ferchichi, A. (2019). Chemical Composition, Antioxidant and Antibacterial Activity of Tunisian Date Palm Seed. Pol. J. Environ. Stud 28: 267-274.

Miller, G.L. (1959). Use of Dinitrosalicylic Acid Reagent for Determination of Reducing Sugar. Anal. Chem 31:426-428.

Montgomery, H. and Dymock, J.F. (1961). The determination of nitrite in water. Analyst 86:414-416.

Mousalamy, A. and Hussein, A. (2016). Aqueous and Methanolic Extracts of Palm Date Seeds and Fruits (Phoenix dactylifera) Protects against Diabetic Nephropathy in Type II Diabetic Rats. Biochemistry & Physiology: Open Access 5:1000205.

National Research Council [NRC] (1993). Nutrient Requirements of Fish. National Academy of Science.

Nkhata, S.G.; Ayua, E.; Kamau, E.H. and Shingiro, J.B. (2018). Fermentation and germination improve nutritional value of cereals and legumes through activation of endogenous enzymes. Food Sci. Nutr 6:2446-2458.

Rahman, M.S.; Kasapis, S.; Al-Kharusi, N.S.Z.; Al-Marhubi, I.M. and Khan, A.J. (2007). Composition characterisation and thermal transition of date pits powders. J. Food Eng 80:1-10.

Repetto, G.; Del Peso, A. and Zurita, J.L. (2008). Neutral red uptake assay for the estimation of cell viability/cytotoxicity.Nat. Protoc 3:1125-1131.

Rinne, M.; Jaakkola, S. and Huhtanen, P. (1997a).Grass maturity effects on cattle fed silage-based diets. 1. Organic matter digestion, rumen fermentation and nitrogen utilization. Anim. Feed Sci. Tech 67:1-17.

Saryono, S. and Proverawati, A. (2019). Hepatoprotective effect of date seeds works through the antioxidant mechanism: a systematic review. Ann. Trop. Med. Public Health 22:301-309.

Shamala, T.R. and Sreekantiah, K.R. (1987). Successive cultivation of selected cellulolytic fungi on rice straw and wheat bran for economic production of cellulases and D-xylanase. Enzyme Microb. Technol 9: 97-101.

Slinkard, K. and Singleton, V.L. (1977). Total Phenol Analysis: Automation and Comparison with Manual Methods. Am J Enol Vitic 28:49.

Sotolu, A.O.; Kigbu, A.A. and Oshinowo, A.J. (2014). Supplementation of date palm (Phoenix dactylifera) seed as feed additive in the diets of juvenile African catfish (Burchell, 1822). J Fish Aquat Sci 9:359-365.

Sumanth, M. and Mamatha, K. (2014). Assessment of learning and memory enhancing activity of phoenix dactylifera (date) in rats. World J. Pharm. Res 3:1215-1226.

Tafti, A.; Dahdivan, N. and Ardakani, S.A. (2017). Physicochemical properties and applications of date seed and its oil. Int. Food Res. J 24:1399-1406.

Tappel, A. and Zalkin, H. (1959). Inhibition of lipide peroxidation in mitochondria by vitamin E. Arch. Biochem. Biophys 80: 333-336.

Theyab, F.; Habib, H.; Othman, A.; Al-Marzooqi, S.; Al-Bawardi, A.; Yasin, J.; Hilary, S.; Souka, U.; Al-Hammadi, S.; Ibrahim, W. and Platat, C. (2017). The antioxidant activity of date seed: Preliminary results of a preclinical in vivo study. Emir. J. Food Agric 29:822-832.

Thouri, A.; Chahdoura, H.; Amira, E.A.; Flamini, G.; Jelled, A. and Hammami, M. (2017). Antioxidant activity, volatile and nonvolatile composition of two Phoenix dactylifera L. by-products. J. Coast. Life Med 5:254-260.

Tibbetts, SM.; Yasumaru, F. and Lemos, D. (2017). In Vitro prediction of digestible protein content of marine microalgae (Nannochloropsis granulata) meals for Pacific white shrimp (Litopenaeus vannamei) and rainbow trout (Oncorhynchus mykiss). Algal Res 21:76-80.

Tumiatti, V.; Bolognesi, M.L.; Minarini, A.; Rosini, M.; Milelli, A.; Matera, R. and Melchiorre, C. (2008). Progress in acetylcholinesterase inhibitors for Alzheimer's disease: an update. Expert Opin Ther Pat 18: 387-401.

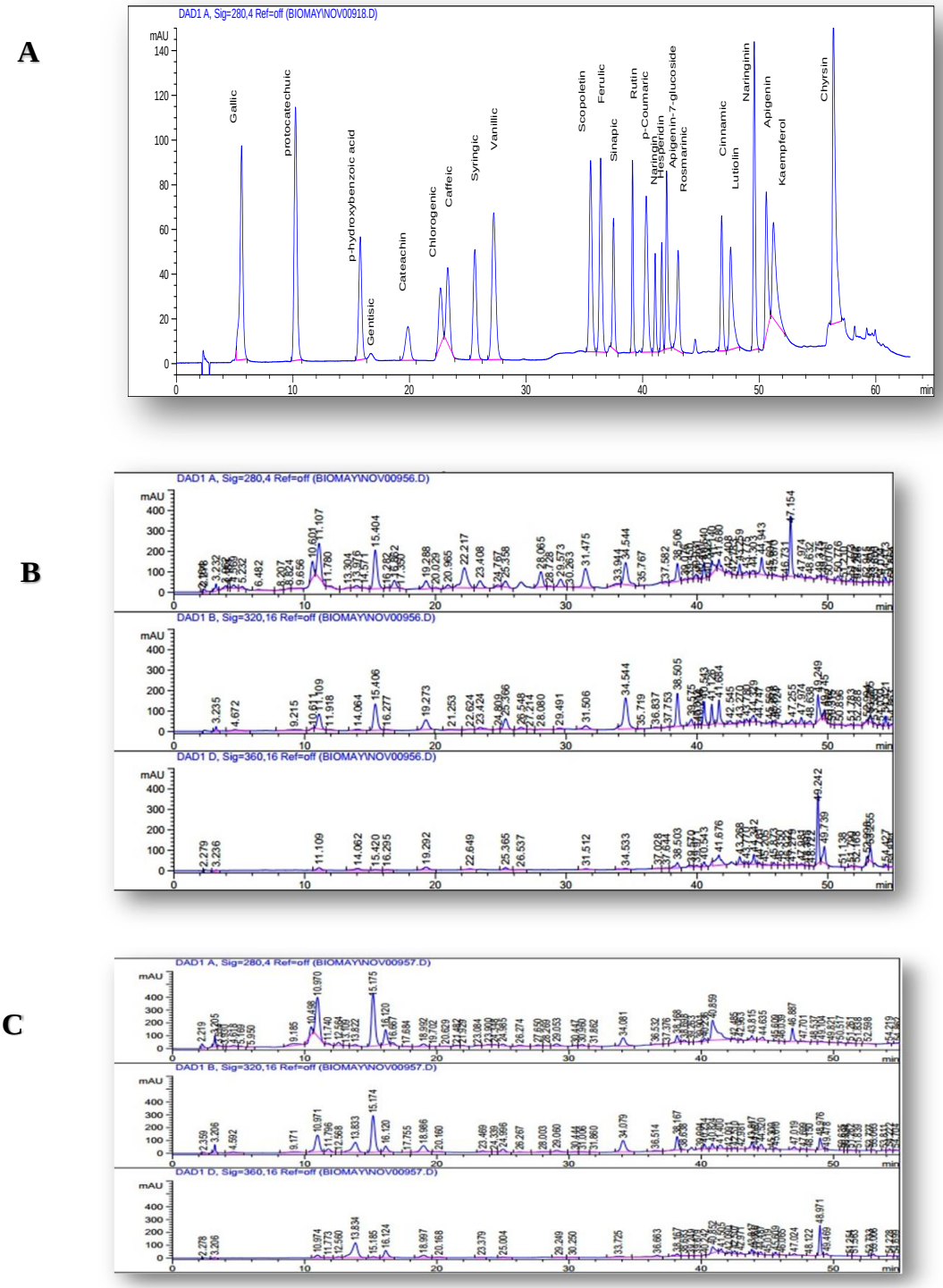
Uckun,Z.; Guzel, S.; Canacankatan, N.; Yalaza, C.; Kibar, D. and Yilmaz, B. (2020). Potential protective effects of naringenin against vancomycin-induced nephrotoxicity via reduction on apoptotic and oxidative stress markers in rats. Drug Chem Toxicol 43:104-111.

Vahedi, A.H.; Hasanpour, M.; Akrami, R. and Chitsaz, H. (2017). Effect of dietary supplementation with ginger (Zingiber officinale) extract on growth, biochemical and hemato-immunological parameters in juvenile beluga (Huso huso). Iran. J. Aquat. Anim. Health 3:26-46.

Wang, X.; Al-Dhalimy, M.; Lagasse, E.; Finegold, M. and Grompe, M. (2001). Liver repopulation and correction of metabolic liver disease by transplanted adult mouse pancreatic cells. Am. J. Clin. Pathol 158: 571-579.

Xiang, H.; Sun-Waterhouse, D.; Waterhouse, G.I.N.; Cui, C. and Ruan, Z. (2019). Fermentation-enabled wellness foods: A fresh perspective. Food Sci. Hum. Wellness 8: 203-243.

Zhang, L.; Liu, Y.; Niu, X.; Liu, Y. and Liao, W. (2012). Effects of acid and alkali treated lignocellulosic materials on cellulase/xylanase production by Trichoderma reesei Rut C-30 and corresponding enzymatic hydrolysis. Biomass and Bioenergy 37:16-24.



Supplementary Figure (1): Typical chromatograms for determination of bioactive compounds in DDP and FDDP ethanolic extracts.

(A) Mixed standards; (B) HPLC chromatogram of DDP ethanolic extract; (C) HPLC chromatogram of FDDP ethanolic extract.