Experimental Evaluation of the Wound-healing and Antioxidant Activities of Tamarind (*Tamarindus indica*) Pulp and Leaf Meal in the African Catfish (*Clarias gariepinus*)

Afrikalı Yayın Balığı (*Clarias gariepinus*) içinde Demirhindi (*Tamarindus indica*) Selüloz ve Yaprak Öğünlerinin Yara İyileşmesi ve Antioksidan Aktivitelerinin Deneysel Olarak Değerlendirilmesi

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Abstract

The fruits and leaves of *Tamarindus indica* have been widely used in traditional medicine for treating wounds and other diseases in Africa. The aim of this study was to investigate the wound-healing property of *T. indica* pulp (TP) and leaf (TL) meal and the importance of antioxidant enzymes in the wound-healing process in the African catfish, *Clarias gariepinus*. Surgical incisions of 10 mm^2 were made aseptically on the lateral part of the catfish, above the pelvic fin and toward the caudal region. The fish were fed experimental diets composed of basal diet fortified with each of TP or TL at concentrations of 0.5%, 1.0%, 1.5%, 2.0%, and 0.0% (untreated) and 0.2% oxytetracycline (treated) as controls in triplicate groups. Changes occurring in the wound area (mm^2) were measured at 3-day-intervals for 15 days. The healing rates and the relative percentage of healing were calculated. Sera collected from the experimental fish were analyzed for oxidative stress biomarkers and antioxidant enzymes. The results showed that fish fed diets treated with TP or TL had significantly faster ($p<0.05$) daily healing rates at the lateral and caudal regions from the 6th to the 12th day compared with those in the control groups. Percentage wound-healing (PWH) at the lateral and caudal regions was significantly enhanced ($p<0.05$) from the 6th day in the tamarind-treated groups. The PWH reached the peak (100%) at the lateral region on the 12th day in fish fed 0.5–2.0% of TL and 1.5%–2.0% of TP diets. Dietary treatment with TP and TL resulted in a lower production of serum malondialdehyde and hydrogen peroxide levels, whereas the reduced glutathione, superoxide dismutase, and glutathione peroxidase levels increased. Fortifying diets of *C. gariepinus* with 1.0%–2.0% of TP and TL meal enhanced wound-healing significantly compared to that of natural healing and with oxytetracycline-fortified diet. The faster wound-healing rate might be a consequence of elevated levels of serum antioxidants in the fish fed tamarind-fortified diets.

Keywords: *Tamarindus indica*, African catfish, experimental study, wound-healing, antioxidant activity, serum antioxidants

Öz

Tamarindus indica'nın meyveleri ve yaprakları, Afrika'daki yaraların ve diğer hastalıkların tedavisi için geleneksel tıpta yaygın olarak kullanılmaktadır. Bu çalışmada, *T. indica* posası (TP) ve yaprak (TL) öğünün yara iyileşme özelliği ve Clarias gariepinus'ta yara iyileşme sürecindeki antioksidan enzimlerin önemi araştırılmıştır. Lateral kısımda, pelvik yüzgecin üzerinde ve kaudal bölgeye doğru aseptik olarak 10 mm^2'lik cerrahi insizyonlar yapılmıştır. Balıklar, her biri TP veya TL ile takviye edilmiş bazal diyetle %0,5, %1,0, %1,5, %2,0 ve %0,0 (müdahale edilmeyen) ve %0,2 oksitetrasiklin (müdahale edilen) kontrol grubu olarak üç grup halinde beslenmiştir. Yara bölgesindeki değişiklikler (mm^2) on beş gün boyunca üç gün aralıklarla ölçülmüştür. İyileşme oranları ve nispi yüzde iyileşmeleri hesaplanmıştır. Deneysel balıklardan alınan serumlar oksidatif stres biyobelirteçleri ve antioksidan enzimler için analiz edilmiştir. Sonuçlar, TP ve TL ile beslenen balıkların, kontrol gruplarına kıyasla, 6. ve 12. günlerde, lateral ve kaudal bölgelerdeki günlük iyileşme oranını anlamlı derecede daha hızlı (p=0,05)

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olduğunu göstermiştir. Lateral ve kaudal bölgelerdeki Yara-İyileşme Yüzdesi (PWH), demirhindi ile tedavi edilen gruplarda 6. günden itibaren önemli ölçüde artmıştır (p=0,05). Tüm TL ve %1,5-2,0 TP diyetleri için PWH 12. günde lateral bölgede doruğa (%100) ulaşmıştır. TP ve TL ile yapılan diyet tedavisi, düşük serum glutatyon, süperoksit dismutaz ve glutatyon peroksidazını arttırırken, serum malondialdehit ve hidrojen peroksidin daha az üretilmesine neden olmuştur. %1,0-2,0 oranında demirhindi küspesi ve yaprak unu ile *C. gariepinus* takviye edici diyetler, doğal iyileşme ve oksietrasiklin takviyeli diyete nispeten önemli ölçüde yara iyileşmesini arttırmıştır. Daha hızlı yara iyileşmesi, balıklarda demirhindi ile takviye edilmiş diyetlerle beslenen serum antioksidanlarının yüksek olmasının bir sonucu olabilir.

Anahtar kelimeler: *Tamarindus indica*, Afrika yayın balığı, deneysel çalışma, yara iyileşmesi, antioksidan aktivite, serum antioksidanları

Introduction

The skin forms the external covering of the body of fish, which protects the fish against mechanical injury and noxious agents. The skin consists of the epidermis and dermal layer. The epidermis of teleost fish consists of fusiform cells, which remain viable and retain the capacity for mitotic division significant for healing processes (Genten et al., 2009; Yang et al., 2015). Skin grows, differentiates and renews itself at all times. A wound is a loss or breaking of cellular and anatomic or functional continuity of living tissues (Ayello, 2005). The closing of an open wound initiates healing because of the responses triggered off by the damaged local cells. Wound-healing is a physiological response of animal to tissue injury which results in replacement of destroyed tissue and restoration of the tissue integrity.

Wound-healing is enhanced by circulation of oxygen and nutrients in wound sites (Abdulla et al., 2009). Although oxygen plays vital roles such as oxidative phagocytosis, synthesis of collagen, angiogenesis and epithelialization in wound-healing, it is also resulted to production of highly reactive oxygen species (ROS) such as free radicals and peroxide, which result to oxidative stress, decelerate these processes and impaired wound-healing. Excessive production of ROS is deleterious to wound-healing (Kanta, 2011; Dunnill et al., 2017), hence, balance between ROS and antioxidants is essential. Antioxidant enzymes play important roles in the detoxification of reactive oxygen metabolites during wound-healing process (Keller et al., 2006; Bryan et al., 2012; Kurahashi and Fujii, 2015).

Plants and their extracts are organic products with immense potential for the management and treatment of wounds. Wound-healing activity of herbal products has been associated with the antimicrobial and antioxidants properties of the phytobiotics (Abdulla et al., 2009, Mohammad et al., 2012; Vifayaraghavan et al., 2017). Plant phytochemical constituents such as tannins, alkaloids and flavonoids contribute to wound-healing activity in animals (Kim et al., 2011; Li et al., 2011; Pawar and Toppo, 2012). Herbal products from *Rafflesia hasseltii* flowers (Abdulla et al., 2009), *Tamarindus indica* (Linn 1753) seed (Mohammad et al., 2012), *Acorus calamus* root and rhizome (Shi et al., 2014) in rat or mice, *Allium cepa* bulb, *Tetracarpidium conophorum* leaf (Bello et al., 2013) and *Azadirachta indica* leaf and oil (Alam et al., 2014) in fish have been used to test the efficacy of herbal products in wound-healing with great potentials.

Tamarindus indica L, commonly called tamarind, is a large tree belonging to the family Leguminoseae (Fabaceae) and subfamily Ceasalpinioideae. Tamarind grows widely in most tropical and subtropical regions of the world (Bhadoriya et al., 2011; Dhamija and Parle, 2012). The bark or leaves of tamarind in the form of powder, decoction, and poultice are applied traditionally on cuts, wounds and abscesses as well as for cleansing wounds caused by guinea worm (Lockett et al., 2000). The ethnomedical use of tamarind in wound-healing in many African countries has been reported (Fabiyi et al., 1993; Diallo et al., 2002; Inngjerdingen et al., 2004; Havinga et al., 2010). Studies have demonstrated the *in vitro* antimicrobial (Gumgumjee et al., 2012, Adeniyi et al., 2017) and antioxidant (Khairunnuur et al., 2009; Lim et al., 2013) activities of tamarind extracts. The role of antioxidant property of tamarind in wound-healing has not been elucidated while scientific information on the wound-healing and *in vivo* antioxidant activities of *T. indica* in fish is limited. Intensive culture of *Clarias gariepinus* (Burchell 1822) is associated with wounds resulting from aggressive behaviours of fish and artificial breeding involving the cutting of testes. This study therefore investigated wound-healing and antioxidant activities of dietary *T. indica* pulp and leaf meal as feed additives in *C. gariepinus*.

Materials and Methods

Plant identification and diets preparation

Fresh tamarind leaves and dried fruits were obtained and authenticated as *Tamarindus indica* Linn with a Voucher Number: UIH-22550. Following the harvest of the plant materials, fresh leaves were removed from the stalk, washed with clean water, drained while the brittle fruit husks were carefully removed and the pulp scraped from the fruits. The leaves were air-dried under shade for 14 days and pulp for 21 days. Both the tamarind pulp (TP) and leaves (TL) were processed into meals. The meals, TP and TL, were included singly at 0.5. 1.0, 1.5 and 2.0% each to fortify the basal diets while 0.0% and 0.2% oxytetracycline (OXY 200 WSP; Kepro, Deventer, Holland) were untreated and treated controls, respectively to make 10 experimental diets.

Experimental fish samples and formation of the wounds

The experimental fish samples consisted of 150 healthy African catfish (*C. gariepinus*). The fish (33.97-45.69g) were randomly selected from each treatment of fish previously fed with the experimental diets in triplicate groups for 12 weeks. Five fish were selected from each replicate and distributed into 50 litre capacity rectangular tanks in triplicates, according to their treatment groups. Following cleaning the portion of the skin with 70% ethanol, surgical incisions wounds of 10 mm^2 were made on each of the fish to the dermis on the lateral part, above the pelvic fin and towards the caudal region (Bello et al., 2013). The fish were returned to the holding tanks (50 litre capacity) and fed the experimental diets at 3% body weight daily. The culture water was changed completely every 48 hours. The water temperature, pH and dissolved oxygen were 26.5±1.00°C, 7.23±0.02 and 5.20±0.50mg/L respectively.

Evaluation of the wound-healing rate

Progressive changes in the wound area were evaluated by measuring the wound area with transparent ruler. The percentage wound-healing (Ammar et al., 2015), daily healing rates (Bell, 2002) and relative percentage healing (Amend, 1981) were calculated using the initial wound area (on 0^{th} day) and areas determined on n^{th} day (n=3, 6, 9, 12, 15) as shown in Table 1.

Table 1. Formulae used for the study

Parameters	Formulae
Percentage wound healing (%)	100 x (Healed area* / Initial wound area)
Daily healing rates (mm^2)	Healed area / Healing time (n^{th} day)
Relative percentage healing (%)	1 – [% wound healing in treatment (on nth day) / % wound healing in untreated control (on nth day)] x 100
Feed Conversion Ratio (FCR)	Feed intake (g) / Weight gain (g)
Survival rate (%)	100 x (Initial fish number - mortality number / Initial fish number)

*Healed area = Initial wound area (on 0^{th} day) – Wound area left (on n^{th} day)

Evaluation of antioxidant activity

After the wound-healing experiment earlier described, blood samples were collected from the caudal vein (Ejraei et al., 2015) of fish sampled from each replicate individually into plain tubes and allowed to clot. The clotted blood samples were centrifuged at 4000 rpm for 10 minutes. Clear sera were collected with micropipette into plain tubes and stored in the freezer until when analysed. The *in vivo* antioxidant properties of dietary tamarind pulp and leaves were evaluated by analysing some oxidative stress biomarkers and antioxidants in the serum of the experimental fish after fifteen days. Concentrations of total protein (Gornal et al., 1949), hydrogen peroxide (Wolff, 1994), malondialdehyde (Varshney and Kale, 1990) as described by Omobowale et al., 2015) Reduced glutathione (Jollow et al. (1974) and activities superoxide dismutase (Oyagbemi et al., 2014) glutathione peroxidase (Rotruck et al., 1973) glutathione-s-transferase (Habig et al., 1974) and myeloperoxidase (Xia and Zweier, 1997) were analyzed spectrophotometrically (Elx800, BioTek, Winooski, USA) using the standard procedures. The whole experimental protocols were performed according to the International (2010/63/EU) and University of Ibadan Institutional rules of animal experiments, clinical studies and biodiversity rights.

Statistical analysis

One-way Analysis of Variance (ANOVA) was used to analyze the data. Duncan multiple range test was used to compare differences among means at 5% probability level using statistical Statistical Analysis System (SAS software, 2010; SAS Institute, Cary, USA).

Results

Wound-healing activity

Fish on diets treated with TP and TL had significantly faster ($p<0.05$) Daily Healing Rates (DHR) at the lateral and caudal re-

Table 2. Daily healing rate (mm^2/day) of surgically wounded *Clarias gariepinus* fed diet treated with tamarind pulp and leaves at three days interval for 15 days

	3^{rd} day		6^{th} day		9^{th} day		12^{th} day		15^{th} day	
Diets	LA	CA	LA	CA	LA	CA	LA	CA	LA	CA
0.0	0.98 ± 0.02^{g}	0.36 ± 0.03^{f}	0.83 ± 0.02^{e}	0.40 ± 0.02^{g}	0.83 ± 0.01^{e}	0.43 ± 0.01^{c}	0.69 ± 0.01^{c}	0.35 ± 0.02^{g}	0.66 ± 0.01^{b}	0.33 ± 0.01^{g}
0.2O	1.19 ± 0.02^{c}	0.53 ± 0.03^{e}	0.85 ± 0.02^{e}	0.49 ± 0.01^{f}	0.84 ± 0.01^{e}	0.44 ± 0.01^{c}	0.78 ± 0.02^{b}	0.37 ± 0.00^{f}	0.67 ± 0.00^{a}	0.33 ± 0.01^{g}
0.5P	1.04 ± 0.01^{f}	0.53 ± 0.02^{e}	0.86 ± 0.01^{e}	0.54 ± 0.02^{de}	0.92 ± 0.01^{d}	0.52 ± 0.02^{b}	0.81 ± 0.05^{ab}	0.44 ± 0.01^{e}	0.67 ± 0.00^{a}	0.37 ± 0.01^{f}
1.0P	1.16 ± 0.02^{de}	0.64 ± 0.03^{d}	0.91 ± 0.01^{cd}	0.53 ± 0.02^{e}	0.98 ± 0.02^{d}	0.54 ± 0.02^{b}	0.82 ± 0.02^{a}	0.47 ± 0.01^{d}	0.67 ± 0.00^{a}	0.42 ± 0.01^{e}
1.5P	1.19 ± 0.02^{c}	0.77 ± 0.02^{c}	0.95 ± 0.02^{b}	0.58 ± 0.01^{c}	1.00 ± 0.02^{b}	0.61 ± 0.02^{ab}	0.83 ± 0.00^{a}	0.54 ± 0.01^{ab}	0.67 ± 0.00^{a}	0.47 ± 0.01^{bc}
2.0P	1.22 ± 0.02^{b}	0.94 ± 0.01^{a}	1.03 ± 0.01^{a}	0.64 ± 0.02^{b}	1.06 ± 0.06^{a}	0.63 ± 0.02^{a}	0.83 ± 0.00^{a}	0.56 ± 0.01^{a}	0.67 ± 0.00^{a}	0.48 ± 0.01^{b}
0.5L	1.33 ± 0.03^{e}	0.52 ± 0.02^{e}	0.89 ± 0.01^{d}	0.56 ± 0.01^{cd}	1.00 ± 0.01^{d}	0.53 ± 0.03^{b}	0.83 ± 0.00^{a}	0.45 ± 0.01^{e}	0.67 ± 0.00^{a}	0.44 ± 0.01^{d}
1.0L	1.16 ± 0.0^{de}	0.56 ± 0.03^{e}	0.92 ± 0.02^{c}	0.63 ± 0.02^{b}	1.01 ± 0.02^{c}	0.59 ± 0.02^{ab}	0.83 ± 0.00^{a}	0.50 ± 0.02^{c}	0.67 ± 0.00^{a}	0.48 ± 0.01^{c}
1.5L	1.18 ± 0.02^{cd}	0.62 ± 0.04^{d}	0.92 ± 0.02^{c}	0.64 ± 0.01^{b}	1.05 ± 0.02^{bc}	0.61 ± 0.01^{ab}	0.83 ± 0.00^{a}	0.53 ± 0.01^{b}	0.67 ± 0.00^{a}	0.47 ± 0.01^{bc}
2.0L	1.27 ± 0.03^{a}	0.84 ± 0.04^{b}	0.95 ± 0.01^{b}	0.71 ± 0.01^{a}	1.06 ± 0.04^{b}	0.68 ± 0.02^{a}	0.83 ± 0.00^{a}	0.54 ± 0.02^{ab}	0.67 ± 0.00^{a}	0.51 ± 0.01^{a}

Means with similar superscripts on the same column are not significantly different at $p<0.05$
LA: lateral; CA: caudal; O: oxytetracycline; P: pulp; L: leaves

Table 3. Percentage wound-healing of surgically wounded *Clarias gariepinus* fed diet treated with tamarind pulp and leaf meal at three days interval for 15 days

	3rd day		6th day		9th day		12th day		15th day	
Diets	**LA**	**CA**	**LA**	**CA**	**LA**	**CA**	**LA**	**CA**	**LA**	**CA**
0.0	29.3±0.5^{g}	10.7±0.7^{f}	50.0±1.0^{e}	24.0±1.0^{g}	74.3±0.6^{e}	38.7±1.1^{e}	83.3±1.1^{c}	42.0±2.0^{g}	98.3±2.1^{b}	49.3±1.1^{g}
0.2 O	35.7±0.7^{c}	16.0±1.0^{e}	50.2±0.7^{e}	27.5±0.5^{f}	75.5±1.8^{e}	39.3±0.6^{e}	93.3±2.9^{b}	44.7±0.6^{f}	99.8±0.4^{a}	49.2±1.4^{g}
0.5P	31.3±0. 3^{f}	16.0±0.5^{e}	51.5±0.5^{e}	32.0±1.0^{e}	82.7±1.1^{d}	46.7±1.5^{d}	96.7±5.8^{ab}	53.0±1.0^{e}	100±0.0^{a}	56.0±2.0^{f}
1.0P	34.7±0.6^{de}	19.3±1.0^{d}	54.7±0.6^{cd}	32.7±1.1^{de}	88.0±2.0^{e}	47.3±1.5^{d}	98.3±2.9^{a}	56.7±1.1^{d}	100±0.0^{a}	62.7±1.5^{e}
1.5P	35.7±0.6^{c}	23.0±0.8^{c}	57.0±1.0^{b}	34.7±0.58^{abc}	90.0±2.0^{bc}	55.3±1.1^{b}	100±0.0^{a}	64.7±1.1^{ab}	100±0.0^{a}	70.0±1.0^{bc}
2.0P	36.7±0.6^{b}	28.1±0.2^{a}	62.0±0.5^{a}	38.7±1.1^{ab}	95.3±5.0^{a}	58.3±1.5^{a}	100±0.0^{a}	67.3±1.1^{a}	100±0.0^{a}	72.5±2.6^{b}
0.5L	34.0±1.0^{e}	15.7±0.4^{e}	53.7±0.6^{d}	33.7±0.6^{a}	90.0±1.0^{bc}	47.3±2.3^{d}	100±0.0^{a}	53.7±1.5^{e}	100±0.0^{a}	66.0±3.5^{ab}
1.0L	34.7±0.3^{de}	16.8±0.7^{e}	55.3±1.5^{c}	38.0±1.0^{ab}	91.3±2.3^{abc}	52.7±1.5^{c}	100±0.0^{a}	60.0±2.0^{c}	100±0.0^{a}	68.5±1.0^{ab}
1.5L	35.3±0.6^{cd}	18.7±0.5^{d}	56.0±1.0^{bc}	38.7±0.6^{abc}	94.5±1.8^{ab}	55.0±1.0^{bc}	100±0.0^{a}	63.0±1.0^{b}	100±0.0^{a}	71.0±2.6^{bc}
2.0L	38.0±1.0^{a}	25.3±1.1^{b}	57.3±1.5^{b}	42.3±0.6^{ab}	96.0±3.61^{a}	57.3±1.5^{ab}	100±0.0^{a}	65.3±2.1^{ab}	100±0.0^{a}	76.00±1.0^{a}

Means with the same letter on the same column are not significantly different at $p<0.05$
LA: lateral; CA: Caudal; OTC: oxytetracycline; P: pulp; L: leaves

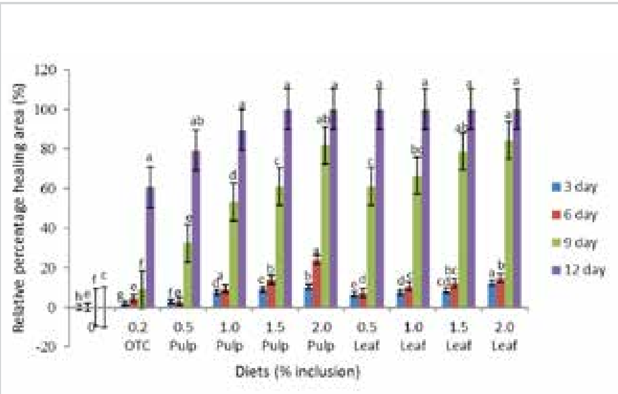

Figure 1. Relative percentage wound healing area at the lateral region of *Clarias gariepinus* fed diets treated with tamarind pulp and leaf meal

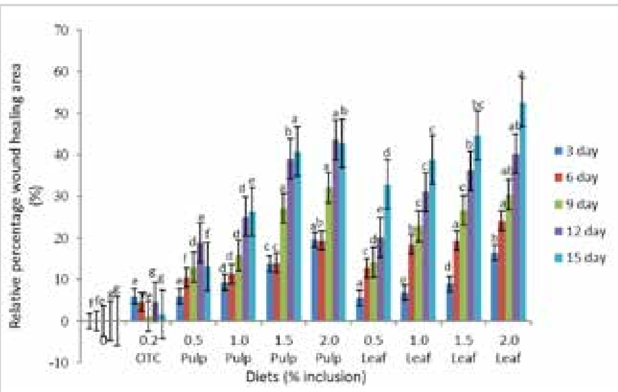

Figure 2. Relative percentage wound healing area at the caudal region of *Clarias gariepinus* fed diets treated with tamarind pulp and leaf meal

gions from the 6th to 12th day compared to the control groups (Table 2). The DHR generally reduced progressively from the 3rd day to the 15th day. Similar to the pattern observed for DHR, Percentage Wound-Healing (PWH) was significantly enhanced ($p<0.05$) from the 6th day in tamarind-treated groups (Table 3). The PWH reached the peak (100%) at the lateral region on the 12th day for all TL and 1.5-2.0% TP groups. The healing pattern at the lateral and caudal regions was dose-dependent. The PWH significantly increased ($p<0.05$) as the levels of inclusion of TP and TL rose.

Treating the diets of *C. gariepinus* with OTC and tamarind enhanced Relative Percentage Wound-Healing (RPWH) at the lateral and caudal region compared to natural healing in untreated control group (Figure 1, 2). On the 9th-15th day (Figure 3-5), fish fed the tamarind-treated diets also demonstrated significantly higher ($p<0.05$) RPWH than those fed the control diets. On the 12th day healing was completed at the lateral region in all the experimental groups except in the control groups and 0.5-1.0% TP group. Healing seemed to be relatively higher in the fish fed TL-treated diets than those fed TP-treated diets.

Other biological indices

During the 15 days study, *C. gariepinus* fed diets fortified with 2.0% TP and 1.0% TL showed significantly lower FCR than those fed untreated control diet. The survival was 100% in all the experimental groups (Table 4).

Antioxidant activity

All tamarind-treated groups showed lower hydrogen peroxide (H_2O_2) than the control groups (Figure 6). Fish fed diets containing 1.5% TP and 2.0% TL had significantly lower ($p<0.05$) H_2O_2 compared to those fed untreated control diet. Treating the diets of *C. gariepinus* with 1.0% TP and 1.0-1.5% TL significantly reduced ($p<0.05$) the sera Malondialdehyde

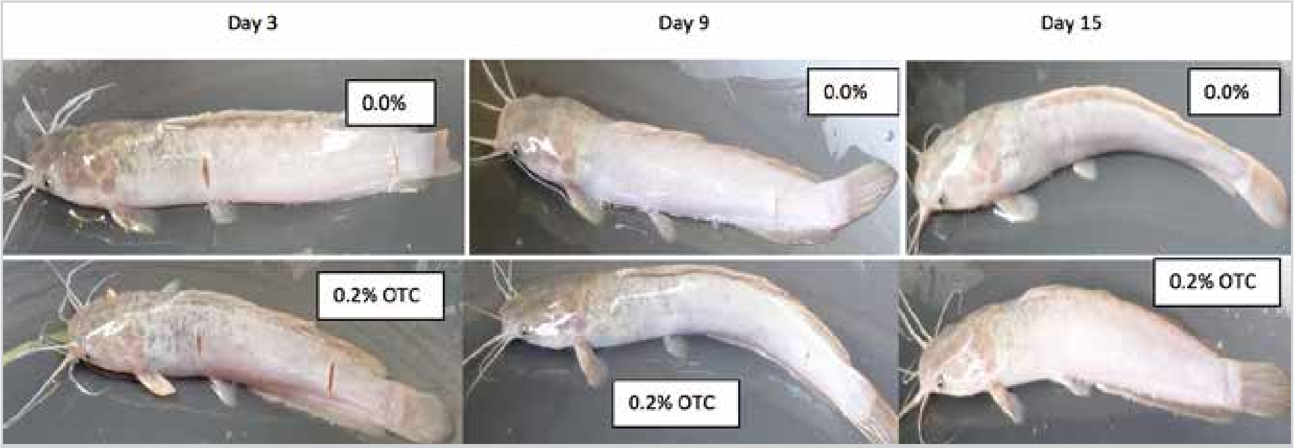

Figure 3. Wound-healing phases in African catfish fed diets fortified with 0.0% (untreated control) and 0.2% oxytetracycline (treated control)

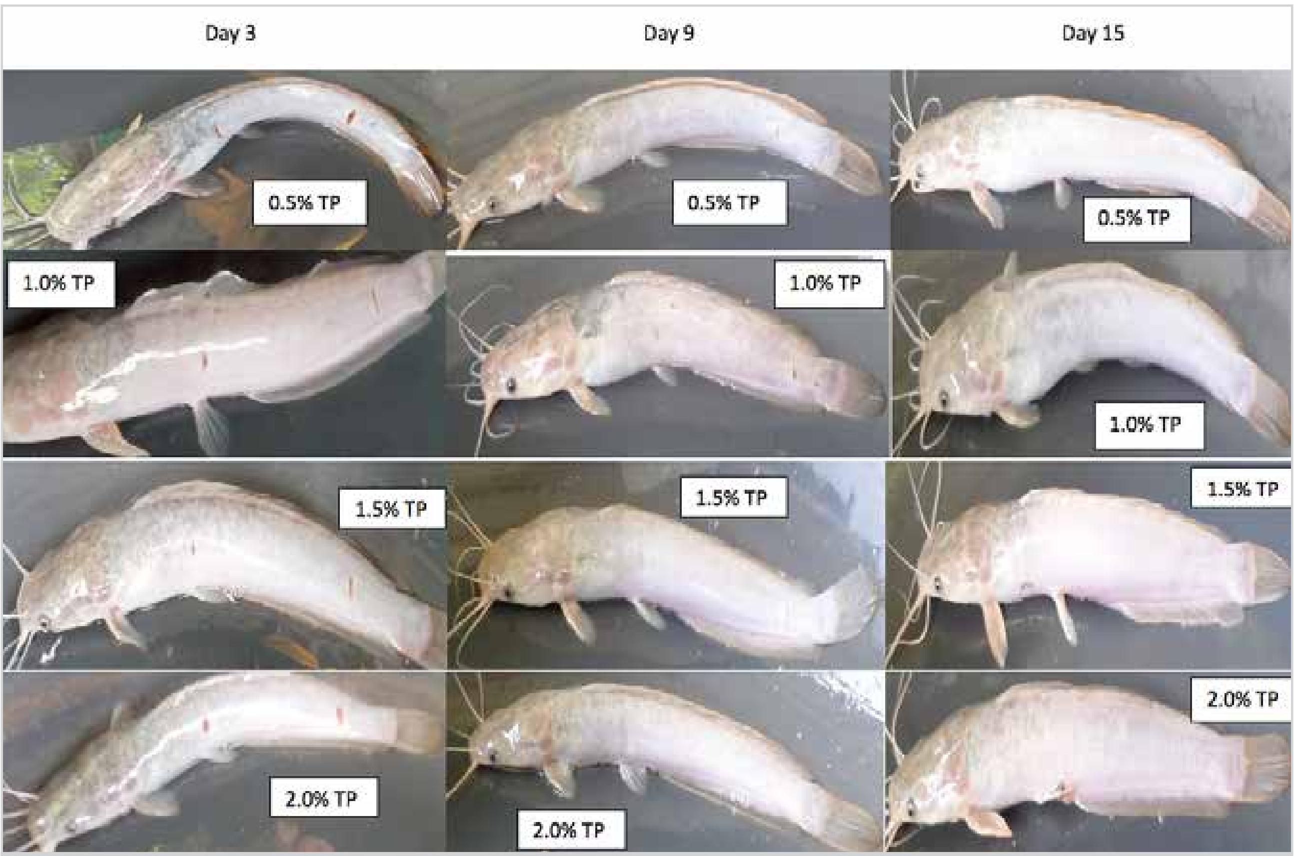

Figure 4. Wound-healing phases in African catfish fed diets fortified with 0.5% - 2.0% TP (tamarind pulp) meal

(MDA) compared to the untreated control diet (Figure 7). The concentration of reduced glutathione (GSH) rose with the increasing level of inclusion of TP and TL in the diets (Figure 8). The concentration of GSH in the sera of fish fed diets treated with 1.0-2.0% TL were significantly higher ($p<0.05$) than the values obtained from the TP-treated and control groups. The activity of Glutathione Peroxidase (GPx) increased significantly ($p<0.05$) in the sera of the groups of fish fed diets

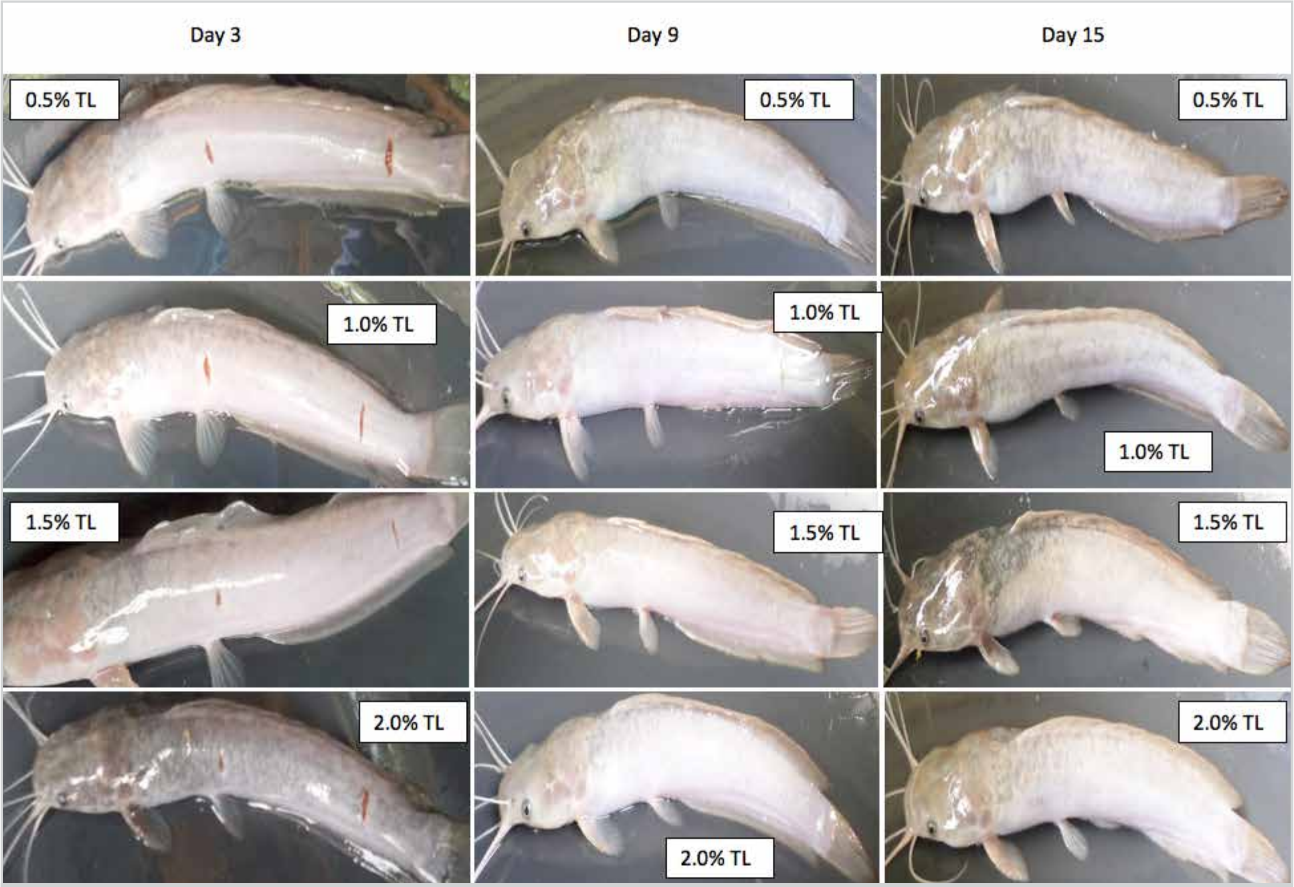

Figure 5. Wound-healing phases in African catfish fed diets fortified with 0.5% - 2.0% TL (tamarind leaf) meal

Table 4. Feed conversion ratio and survival of experimentally wounded *Clarias gariepinus* fed diets fortified with varying levels of *Tamarindus indica* pulp and leaves for 15 days

Treatments	Feed conversion ratio	Survival (%)
0.00%	1.64^{a}	100
0.20% Oxytetracycline	1.48^{ab}	100
0.50% Pulp	1.37^{ab}	100
1.00% Pulp	1.36^{ab}	100
1.50% Pulp	1.24^{ab}	100
2.00% Pulp	1.13^{b}	100
0.50% Leaves	1.40^{ab}	100
1.00% Leaves	1.13^{b}	100
1.50% Leaves	1.34^{ab}	100
2.00% Leaves	1.36^{ab}	100

containing 1.5-2.0% TP compared to the untreated control group. Groups of fish fed TP-treated diets exhibited higher activity of Superoxide Dismutase (SOD) than those fed control and TL-treated diets (Figure 9).

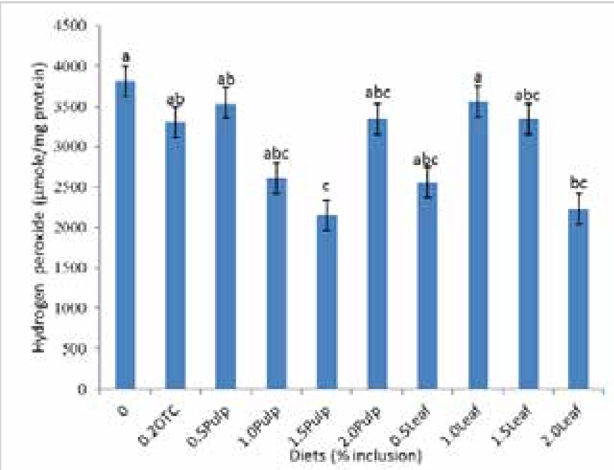

Figure 6. Sera hydrogen peroxide of *Clarias gariepinus* fed diets treated with tamarind pulp and leaf meal

Although, TP-treated groups showed higher activity of Gluthathione-S-Transferase (GST) compared to OTC-treated group, the values did not differ significantly (p>0.05). Contrary to the observation with GST activity in TP groups,

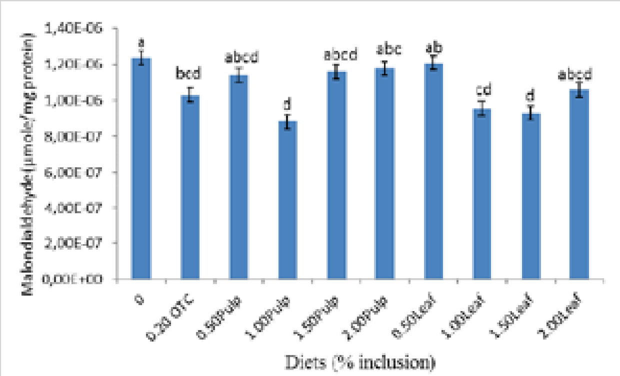

Figure 7. Sera Malondialdehyde of *Clarias gariepinus* fed diet treated with tamarind pulp and leaf meal

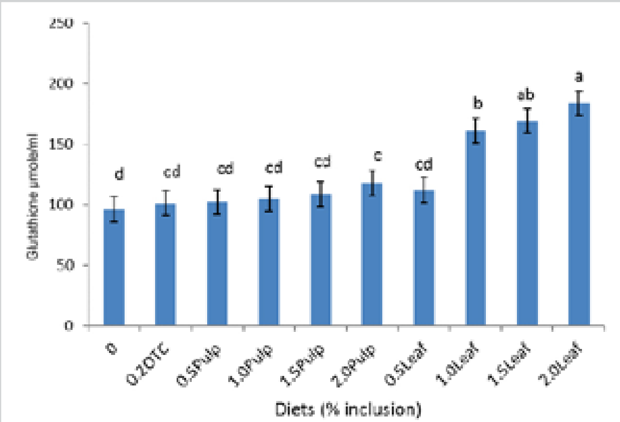

Figure 8. Sera reduced glutathione of *Clarias gariepinus* fed diets treated with tamarind pulp and leaf meal

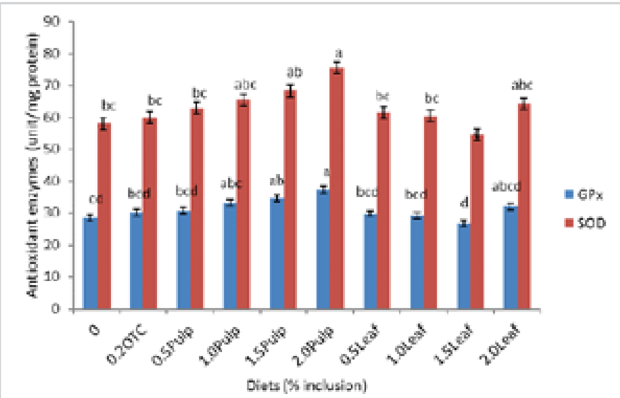

Figure 9. Activities of sera Glutathione Peroxidase (GPx) and Superoxide Dismutase (SOD) of *Clarias gariepinus* fed diets treated with tamarind pulp and leaf meal

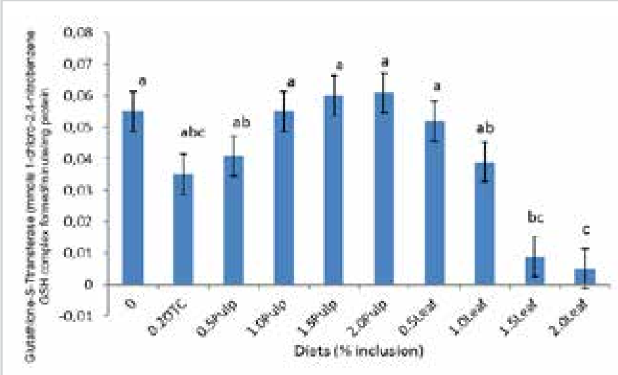

Figure 10. Activity of sera glutathione-S-transferase of *Clarias gariepinus* fed diets treated with tamarind pulp and leaf meal

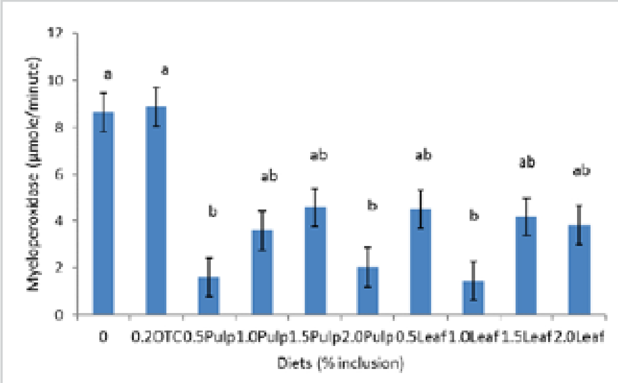

Figure 11. Activity of sera myeloperoxidase of *Clarias gariepinus* fed diets treated with tamarind pulp and leaf meal

the activity of GST decreased with increasing level of TL in the diet (Figure 10). Figure 11 shows that fish on control diets had higher Myeloperoxidase (MPO) activity, than those on diets containing TP and TL. The activity of MPO did not differ significantly ($p>0.05$) among the tamarind-treated groups.

Discussion

This study investigated the wound-healing and antioxidants activities of *Tamarindus indica* pulp and leaf meal and related possible role of natural antioxidants in wound-healing. Faster wound-healing was observed at three-day intervals in groups of wounded *C. gariepinus* fed with tamarind-treated diets compared to the group on OTC-treated and untreated control diets.

The faster wound-healing observed in fish fed OTC-treated diets, compared to the untreated control, is similar to observation on faster healing of wounds / lesions treated with OTC earlier reported (Chandler et al., 2010; Ajith et al., 2016). The faster healing obtained from the fish fed tamarind-treated diets confirms the efficacy of this plant in traditional wound-healing. The bark or leaves of tamarind have been reported to be used traditionally for healing wounds (Fabiyi et al., 1993; Lockett et al., 2000; Havinga et al., 2010). The healing activity of tamarind pulp and leaves in the diets of *C. gariepinus* might be due to the ability of the phytochemical in these herbal products to promote formation of collagen. Collagen is the principal component of connective tissue, which plays a key role in tissue regeneration (Cohen et al., 1992; Abdulla et al., 2009).

Better wound-healing rate was similarly observed in *C. gariepinus* fed diets containing walnut leaf and onion bulb (Bello et al., 2013). Alam et al. (2014) reported enhanced wound-healing in fish on kalojira seed oil, neem seed oil and leaves extract compared to control diets. Inclusion of extracts of *Rafflesia hasseltii* in the diets of Sprague Dawley rat has also been proved to enhance wound-healing (Abdulla et al., 2009). Shi et al. (2014) further reported higher wound-healing rate in mice fed with diets treated with *Acorus calamus* extracts compared to the control diet.

Antioxidants in phytobiotics have been reported to promote wound-healing activity in animals (Abdulla et al., 2009; Mohammad et al., 2012). Reduction in the biomarkers of oxidative stress and complementary higher activities of sera GSH, GPx and SOD in *C. gariepinus* on dietary tamarind demonstrated antioxidant ability of TP and TL. Spontaneous dismutation of superoxide radicals to H_2O_2 and less reactive oxygen is enhanced by SOD while GPx remove it in the presence of GSH as substrate (Kohen and Nyska, 2002). The antioxidant activities demonstrated might be due to flavonoid in the tamarind pulp and leaves (Adeniyi et al., 2017) resulting to the enhanced wound-healing of the fish. Dietary flavonoid in animal has been recognized for antioxidants activities (Yao et al., 2004).

The activity of GST was not seriously affected except the significant reduction at 2.0% TL inclusion level. Cell inflammation and oxidative stress have been associated with increased MPO activities, as high MPO may be released from neutrophil when reactive oxygen species is high (Akinrinde et al., 2015). Therefore, the reduction in MPO activity in the tamarind-treated *C. gariepinus* demonstrated the chemoprotective effects of the tamarind additives and this might have contributed to faster healing observed in fish fed diets fortified with tamarind pulp and leaf meal.

Similar antioxidant properties of some phytobiotics have been reported: Activity of SOD was similarly higher in common carp and prawn fed diets containing 0.1-0.2% *Rheum officinale* anthraquinone extract than those fed control diet (Xie et al., 2008; Liu et al., 2010). Giannenas et al. (2012) also observed decreased MDA and increased GSH-based enzymes in the fillet of rainbow trout fed diets containing thymol and carvacrol. Furthermore, SOD activity was increased in pacific red snapper fed microalgae (Reyes-Becerril et al., 2014) and in Nile tilapia on diets supplemented with *Astragalus* polysaccharides (Zahran et al., 2014). The later authors however reported insignificant effect on MDA.

The possible mechanism of the enhanced wound-healing of *Clarias gariepinus* fed with diets fortified with tamarind in this study might be reduction of oxidative stress. Utilization of antioxidants has been reported to enhance repair of tissues and wound-healing (Fitzmaurice et al., 2011; Shetty, 2013; Kurahashi and Fujii, 2015). Elevated levels of MDA and hydrogen peroxide accompanied with higher production of antioxidant levels seemed to enhance wound-healing in this study. Low levels of antioxidants and elevated levels of markers of oxidative stress have been reported to delay wound-healing due to damage to cellular membranes, proteins and lipids (Rasik and Shukla, 2000).

In conclusion, this study revealed the *in vivo* antioxidant activity of *Tamarindus indica* pulp and leaves and its utilization as proven wound-healing agent. These natural antioxidants might have been responsible for enhanced wound-healing observed in *Clarias gariepinus*. Utilization of 1.0-2.0% air-dried *Tamarindus indica* pulp and leaf meal as feed additives significantly enhanced wound-healing in *Clarias gariepinus* and it is therefore recommended for use.

Ethics Committee Approval: Ethics committee approval was received for this study from the ethics committee of University of Ibadan, Nigeria.

Peer-review: Externally peer-reviewed.

Author Contributions: Concept – O.V.A.; Design – O.V.A., F.E.O., B.O.E.; Supervision – F.E.O., B.O.E.; Resources – O.V.A., A.A.O.; Materials – O.V.A., A.A.O.; Data Collection and/or Processing – O.V.A.; Analysis and/or Interpretation – O.V.A.; Literature Search – O.V.A.; Writing Manuscript – O.V.A.; Critical Review – O.V.A., F.E.O., B.O.E., A.A.O.

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