WOUND HEALING OF AFRICAN CATFISH (*Clarias gariepinus*) FED A COMBINATION OF PLANT–BASED HERBAL SUPPLEMENTED DIETS

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Abstract

This study evaluated the wound healing effect of medicinal plants mixture (MPLM) fenugreek (*Trigonella foenum-graecum*), scent leaf (*Ocimum gratissimum)*, stone breaker (*Phyllanthus niruri*), and garlic (*Allium sativum*) dietary supplementation on *Clarias gariepinus.* One hundred and forty male and female *C. gariepinus* were divided into 7 treatments, including the control and 6 treatment groups in two replications, 10 *C. gariepinus* per treatment (5 males and 5 females). Seven (7) experimental diets composed of Control (0%) $MPLM_2$ (0.5%), $MPLM_3$ (1.0%) $MPLM_4$ (1.5%), $MPLM_5$ (2.0%), $MPLM_6$ (2.5%), $MPLM_7$ (3.0%) was fed twice daily at 3% body weight of 40% crude protein for 14 days in 14 experimental tanks (1.8 m x 1.8 m x 1.2 m). The 1 cm^2 incision was made on the *C. gariepinus* below the dorsal fin above the lateral line, and the wound closure percentage healing, and daily healing rate on the lateral parts of the male and female *C. gariepinus* were investigated at 0, 7, 10 and 14 days. The results of the study revealed that wound closure, percentage healing and daily healing rate were better in the treated groups compared to the control. Also, the results obtained showed that the male *Clarias gariepinus* had better healing compared to the female. The histology of the male and female muscle wound closure of *C. gariepinus* revealed that slight observable changes were observed in the control group and MPLM 2 and control group, MPLM 2 (0.5%) and MPLM 3 (1.0%) for male and female *C. gariepinus*, respectively. However, no visible structural changes were observed among the other dietary groups. Diets with medicinal plants had healing activity, and their inclusion in fish feed promotes wound healing in *Clarias gariepinus.*

Keywords: Medicinal plants mixture; *Clarias gariepinus;* Dermal wound healing; Histology; Microscopic examinations

Introduction

Fish is a major source of animal protein to humans (Ineyougha *et al*., 2015; Izah and Angaye, 2015; Angaye *et al*., 2015) and have been widely employed in other downstream applications, including feed production. The aquatic animal skin protects the tissues and organs from physical, mechanical, chemical and microbial damage, and they mainly consist of the epidermis, dermis and subcutaneous tissue (Aghoghovwia *et al*., 2018). Wound infection is one of the health concerns that is caused and aggravated by the invasion of pathogenic microorganisms (Mama *et al*., 2014). Cutaneous wound is the most common disorder in fish that significantly increases morbidity and mortality due to access of various pathogens and predators to underlying tissues (Coutant *et al*., 2019). Injury sustained by aquatic organisms often causes disruption of neutrophils, monocytes and macrophages at the site of injury (Barrientos *et al*., 2008; Akunne *et al*., 2016).

Cultured fish encounter and suffer from superficial injuries and different stressors as a result of intensification in fish farms (Brown *et al*., 2018; Reyes, 2019). Literature has revealed the uses of medicinal plants and their bioactive principles or extracts for various purposes in aquaculture farms. They exhibited various prophylactic properties such as growth-promoting, antimicrobial, immunostimulant, antioxidants, and hepatoprotective activities, appetite and immune stimulation, and anti-stress properties are some of the biological activities of medicinal plants in aquatic organisms (Citarasu, 2010; Chakraborty and Hancz, 2011; Awad and Awaad, 2017).

Also, Jinendiran *et al.* (2019) reported that healing agents must be available in wound closure, and the dietary supplementation of medical plants acts as an alternative to synthetic agents. Considering the sequence and frequency of skin injuries of fish, such as abrasions and bruises, in the culture system during different breeding systems caused by manipulations, transportation, density, parasitic factors, etc. The wound healing effects of a mixture of medicinal plants in *C. gariepinus* have not been widely and fully investigated. The present study investigates the efficacy of oral use of a mixture of medicinal plants as a feasible and less stressful method in the healing process of skin incisional wounds of *C. gariepinus*.

Materials and Methods

Plant Collection, Identification and Preparation

Fenugreek (*Trigonella foenum-graecum*) and garlic (*Allium sativum*) were obtained from Mooju Ventures, Lagere, Ile–Ife, Osun State, Nigeria. Scent leaf (*Ocimum gratissimum)* and stone breaker (*Phyllanthus niruri*) were obtained from Ondo city and Igbodan-Lisa, Okitipupa, respectively. These plant-based medicinal plants were identified at the Department of Biological Sciences (Botany Programme) of Olusegun Agagu University of Science and Technology, Okitipupa. The scent leaf and stone breaker were air-dried for four weeks. All the plants were ground into fine powder using a fabricated hammer mill and stored (4 ^{0}C) until required.

Experimental Fish and Design

A total of 140 healthy *C. gariepinus (*850±20 g) were bought and transferred to the Fisheries and Aquaculture Technology Laboratory of Olusegun Agagu University of Science and Technology, Okitipupa. The *C. gariepinus* were considered free of diseases by general observation and microscopic wet mount examination of skin investigations before the commencement of the experiment. The fish were acclimatized in rectangular experimental tanks for 30 days and fed with Coppens at three per cent of body weight. One hundred and forty *C. gariepinus* (850±20 g) were divided randomly into seven treatments of 10 fish per treatment (5 males and 5 females).

The experiment lasted for 2 weeks, during which the fish were fed at 3% body weight daily. The diet per day was divided into two: 1.5% given in the morning by 8.00 – 9.00 and 1.5% in the evening by 5.00 pm.

Formulation and Preparation of Experimental Diet

Seven experimental diets were formulated at 40% crude protein, 14.67% ash, 10.45% crude fibre, 7.46% ether extract, 6.31% moisture, and 21.10% nitrogen-free extract using Pearson's square method to determine individual ingredient contribution at g/100g diet. Each ingredient, such as fish meal, soybean, blood meal, maize, wheat bran, vitamin-mineral premix, starch, vegetable oil, and Di-calcium phosphate, was weighed using a sensitive weighing balance. Medicinal plants mixture (MPLM) {fenugreek (*Trigonella foenum-graecum*), scent leaf (*Ocimum gratissimum)*, stone breaker (*Phyllanthus niruri*), and garlic (*Allium sativum*)} was thoroughly mixed in a ratio of 1: 0.5: 0.5: 0.5 on a dry *w*/*w* basis respectively. This combination was decided based on previous findings. The ground medicinal plants mixture was added at different inclusion levels of 0.5, 1.0, 1.5, 2.0, 2.5 and 3.0%. The dry ingredients were mixed thoroughly using the manual method. The mixture of plant ingredients was added as a feed additive in the study as a partial replacement for the vitamin-mineral premix. Each diet was treated separately. Water was added, and the result was a dough that was pelleted through a 2 mm pelleting machine to form a noodle-like strand, which was manually broken into suitable sizes for the *Clarias gariepinus*. The pelleted diets were oven-dried at 60 ^{0}C and stored in airtight nylon bags at room temperature to prevent mycotoxin formation until required.

Skin Wounding Procedure

Clarias gariepinus were fasted for 24 hours before anaesthetized with 2-phenoxy ethanol. Each fish was placed on a wooden table for the wounding procedure, and the lateral parts of the fish were aseptically prepared (medical examinations or procedures that prevent contamination or infection of microbes). A standard template (a transparent paper, drawing the site on a well-uniform 1 mm cubic square quadrant) was placed on the fish, and the cuts were made on the lateral part of the fish to draw the wound area on the fish's body (Bello *et al.*, 2013). The fish were taken out of the water every day to examine the wound closure. Photograph and measurement of wound closure were done using a measuring tape rule at 0, 7, 10 and 14 days in the wound area in two-dimensional healing. Percentage healing and daily healing rate were calculated as;

$$\% \text{ healing} = \frac{\text{change in wound area (area of regenerated tissue)}}{\text{initial wound area}} \times 100$$

$$\text{Daily healing rates} = \frac{\text{total percentage of area healed}}{\text{number of days' measurement taken}}$$

(Bello *et al.*, 2013)

Histopathological Examination

The muscle wound closure of male and female *C. gariepinus* from the control and treated groups were studied and compared for histopathological changes. The male and female (n = 2) were obtained from each treatment, dissected and immediately fixed in 10% formalin solution for 24 hours (Olusola *et al*. 2023; Bello *et al*., 2013). At the end of 24 hours, tissue samples were washed in running water to remove traces of formalin. Specimens were dehydrated by passing through a graded series of alcohol (30%, 50%, 70%, 95% and absolute ethanol) for two hours each. Specimens were later passed through xylene (clearing agent) to remove the alcohol, and molten paraffin wax was used to impregnate the tissues in a vacuum oven at 56 °C and allowed to solidify following the procedures of Olusola *et al*., (2021). The tissue blocks were cut by trimming and attaching them to wood blocks in preparation for sectioning. Sectioning and staining of tissues were done according to Olusola *et al*. (2023). A light photomicroscope attached to a 35 mm camera was used to examine the tissue sections.

Determination of Phytochemical Screening of *Trigonella foenum-graecum*, *Ocimum gratissimum*, *Phyllanthus niruri*, and *Allium sativum*

The phytochemical screening of *Trigonella foenum-graecum*, *Ocimum gratissimum*, *Phyllanthus niruri*, and *Allium sativum* such as saponins, tannins, steroids, terpenoids, alkaloids, polyphenols, anthocyanin, flavonoids, phenols, and phytate were obtained using qualitative techniques as described by Olusola *et al*., (2024).

Statistical Analysis

Data were statistically analyzed using one–way analysis of variance (ANOVA), using SPSS version 20. Duncan's Multiple comparisons among means were made when significant F-values were observed at P = 0.05.

Results

The results of phytochemical screening of stone breakers, scent leaves, fenugreek and garlic revealed the presence of alkaloids, flavonoids, saponins, protein, phenols, steroids, glucosinolates and tannins in garlic, fenugreek seeds and scent leaves, while saponins, phenols, steroids and tannins were absent in the stone breakers. Also, glucosinolates and garlic were absent in scent leaves and garlic, respectively (Table 1).

Table 1: Phytochemical screening of stone breakers, scent leaves, fenugreek and garlic

	Stone breakers	Scent leaves	Fenugreek seeds	Garlic
Alkaloids	+	+	+	+
Flavonoids	++	++	+	+
Saponins	-	++	+	+++
Protein	+	+	+	+++
Phenol	-	+	+	-
Steroids	-	+++	+	++
Glycosinate	++	-	+	++
Tannin	-	+++	+	-+

+++ = Present and available in abundant quantity, ++ = Moderate quantity, + = Small quantity, - = Absent,

Wound healing experiment of male and female Clarias gariepinus fed medicinal plant mixture supplemented diets for 14 days.

The results of wound healing of *C. gariepinus* on the lateral part showed better wound closure in the medicinal plant mixture-supplemented diets compared to the control. The results revealed that percentage healing and daily healing rate were the best in MPLM 5 (98.00; 14.00) and (99.00, 14.14) for the male and female *C. gariepinus* at 7 days respectively, while the lowest was recorded in the control (80.00, 11.48) (84.00,12.00) for male and female *C. gariepinus* respectively. The results of wound closure at 10 days show the highest wound closure in MPLM 3 – MPLM 5 for male and female, while the least was recorded in control (96.00, 9.60) (96.33,9.63) for percentage healing and daily healing rate at 14 days respectively. All the treatments recorded complete wound closure of 100% and 7.14% for percentage healing and daily healing rate for male and female *C. gariepinus*, respectively (Table 2 - 4).

The mean values in each column with similar superscript are not significantly different (P> 0.05)

Table 2: Wound healing of male and female *C. gariepinus* fed a medicinal plant mixture at 7 days

Table 3: Wound healing of male and female *C. gariepinus* fed medicinal plant mixture at 10 days

	Male (10 days)				Female (10 days)			
	Initial wound area (cm^2)	Change in wound area (cm^2)	% Healing	Daily healing rate	Initial wound area (cm^2)	Change in wound area (cm^2)	% Healing	Daily healing rate
Control	1.00	0.04 ± 0.01^{a}	96.00 ± 0.58^{a}	9.60 ± 0.05^{a}	1.00	0.04 ± 0.01^{a}	96.33 ± 0.67^{a}	9.63 ± 0.07^{a}
MPLM2	1.00	0.01 ± 0.00^{a}	99.00 ± 1.00^{b}	9.90 ± 1.00^{b}	1.00	1.00 ± 0.00^{b}	100.00 ± 0.00^{c}	10.00 ± 0.00^{c}
MPLM3	1.00	1.00 ± 0.00^{b}	100.00 ± 0.00^{b}	10.00 ± 0.00^{b}	1.00	1.00 ± 0.00^{b}	100.00 ± 0.00^{c}	10.00 ± 0.00^{c}
MPLM4	1.00	1.00 ± 0.00^{b}	100.00 ± 0.00^{b}	10.00 ± 0.00^{b}	1.00	1.00 ± 0.00^{b}	100.00 ± 0.00^{c}	10.00 ± 0.00^{c}
MPLM5	1.00	1.00 ± 0.00^{b}	100.00 ± 0.00^{b}	10.00 ± 0.00^{b}	1.00	1.00 ± 0.00^{b}	100.00 ± 0.00^{c}	10.00 ± 0.00^{c}
MPLM6	1.00	0.01 ± 0.00^{a}	99.33 ± 0.33^{b}	9.93 ± 0.03^{b}	1.00	0.01 ± 0.00^{a}	99.67 ± 0.33^{c}	9.97 ± 0.33^{c}
MPLM7	1.00	0.01 ± 0.00^{a}	98.67 ± 0.67^{b}	9.87 ± 0.07^{b}	1.00	0.02 ± 0.00^{a}	98.00 ± 0.00^{b}	9.80 ± 0.00^{b}

The mean values in each column with similar superscript are not significantly different (P> 0.05)

Table 4: Wound healing of male and female *C. gariepinus* fed medicinal plant mixture at 14 days

	Male (14 days)				Female (14 days)			
	Initial wound area (cm^2)	Change in wound area (cm^2)	% Healing	Daily healing rate	Initial wound area (cm^2)	Change in wound area (cm^2)	% Healing	Daily healing rate

	Male (7 days)				Female (7 days)			
	Initial wound area (cm^2)	Change in wound area (cm^2)	% Healing	Daily healing rate	Initial wound area (cm^2)	Change in wound area (cm^2)	% Healing	Daily healing rate
Control	1.00	0.20 ± 0.03^{c}	80.00 ± 2.89^{a}	11.48 ± 0.41^{a}	1.00	0.16 ± 0.04^{a}	84.00 ± 4.10^{a}	12.00 ± 0.57^{a}
MPLM2	1.00	0.16 ± 0.03^{bc}	84.00 ± 2.65^{ab}	12.00 ± 0.38^{ab}	1.00	0.05 ± 0.02^{a}	95.33 ± 1.76^{a}	13.62 ± 0.25^{a}
MPLM3	1.00	0.06 ± 0.01^{ab}	94.33 ± 1.45^{bc}	13.48 ± 0.21^{bc}	1.00	0.22 ± 0.01^{a}	78.00 ± 21.00^{a}	11.14 ± 3.00^{a}
MPLM4	1.00	0.11 ± 0.04^{abc}	89.33 ± 3.71^{abc}	12.76 ± 0.53^{abc}	1.00	0.08 ± 0.03^{a}	92.33 ± 2.98^{a}	13.19 ± 0.42^{a}
MPLM5	1.00	0.02 ± 0.01^{a}	98.00 ± 1.00^{c}	14.00 ± 0.14^{c}	1.00	0.10 ± 0.00^{a}	99.00 ± 0.00^{a}	14.14 ± 0.00^{a}
MPLM6	1.00	0.06 ± 0.05^{ab}	93.67 ± 4.84^{bc}	13.38 ± 0.69^{bc}	1.00	0.16 ± 0.49^{a}	84.00 ± 4.93^{a}	12.00 ± 0.71^{a}
MPLM7	1.00	0.14 ± 0.06^{abc}	86.00 ± 6.08^{abc}	12.29 ± 0.87^{abc}	1.00	0.04 ± 0.01^{a}	96.00 ± 1.00^{a}	13.72 ± 0.14^{a}
Control	1.00	1.00 ± 0.00^{a} (with mild scar)	100.00 ± 0.58^{a}	7.14 ± 0.00^{a}	1.00	1.00 ± 0.00^{a} (with mild scar)	100.00 ± 0.00^{a}	7.14 ± 0.00^{a}
MPLM2	1.00	1.00 ± 0.00^{a}	100.00 ± 0.00^{a}	7.14 ± 0.00^{a}	1.00	1.00 ± 0.00^{a}	100.00 ± 0.00^{a}	7.14 ± 0.00^{a}
MPLM3	1.00	1.00 ± 0.00^{a}	100.00 ± 0.00^{a}	7.14 ± 0.00^{a}	1.00	1.00 ± 0.00^{a}	100.00 ± 0.00^{a}	7.14 ± 0.00^{a}
MPLM4	1.00	1.00 ± 0.00^{a}	100.00 ± 0.00^{a}	7.14 ± 0.00^{a}	1.00	1.00 ± 0.00^{a}	100.00 ± 0.00^{a}	7.14 ± 0.00^{a}
MPLM5	1.00	1.00 ± 0.00^{a}	100.00 ± 0.00^{a}	7.14 ± 0.00^{a}	1.00	1.00 ± 0.00^{a}	100.00 ± 0.00^{a}	7.14 ± 0.00^{a}
MPLM6	1.00	1.00 ± 0.00^{a}	100.00 ± 0.00^{a}	7.14 ± 0.00^{a}	1.00	1.00 ± 0.00^{a}	100.00 ± 0.00^{a}	7.14 ± 0.00^{a}
MPLM7	1.00	1.00 ± 0.00^{a}	100.00 ± 0.00^{a}	7.14 ± 0.00^{a}	1.00	1.00 ± 0.00^{a}	100.00 ± 0.00^{a}	7.14 ± 0.00^{a}

The mean values in each column with similar superscript are not significantly different (P> 0.05)

Histological parameters of male and female C. gariepinus fed a medicinal plant mixture.

There were slight observable changes in the female muscle wound closure of *C. gariepinus* among all the treatment groups except the MPLM 4, MPLM 6 and MPLM 7. Also, the MPLM 3 – MPLM 7 recorded no visible abnormal structural changes in the male muscle wound closure, but slight observable changes were observed in the control group and MPLM 2. Results of the histological study were shown in Plates 1 (female *C. gariepinus*) and 2 (male *C. gariepinus)*.

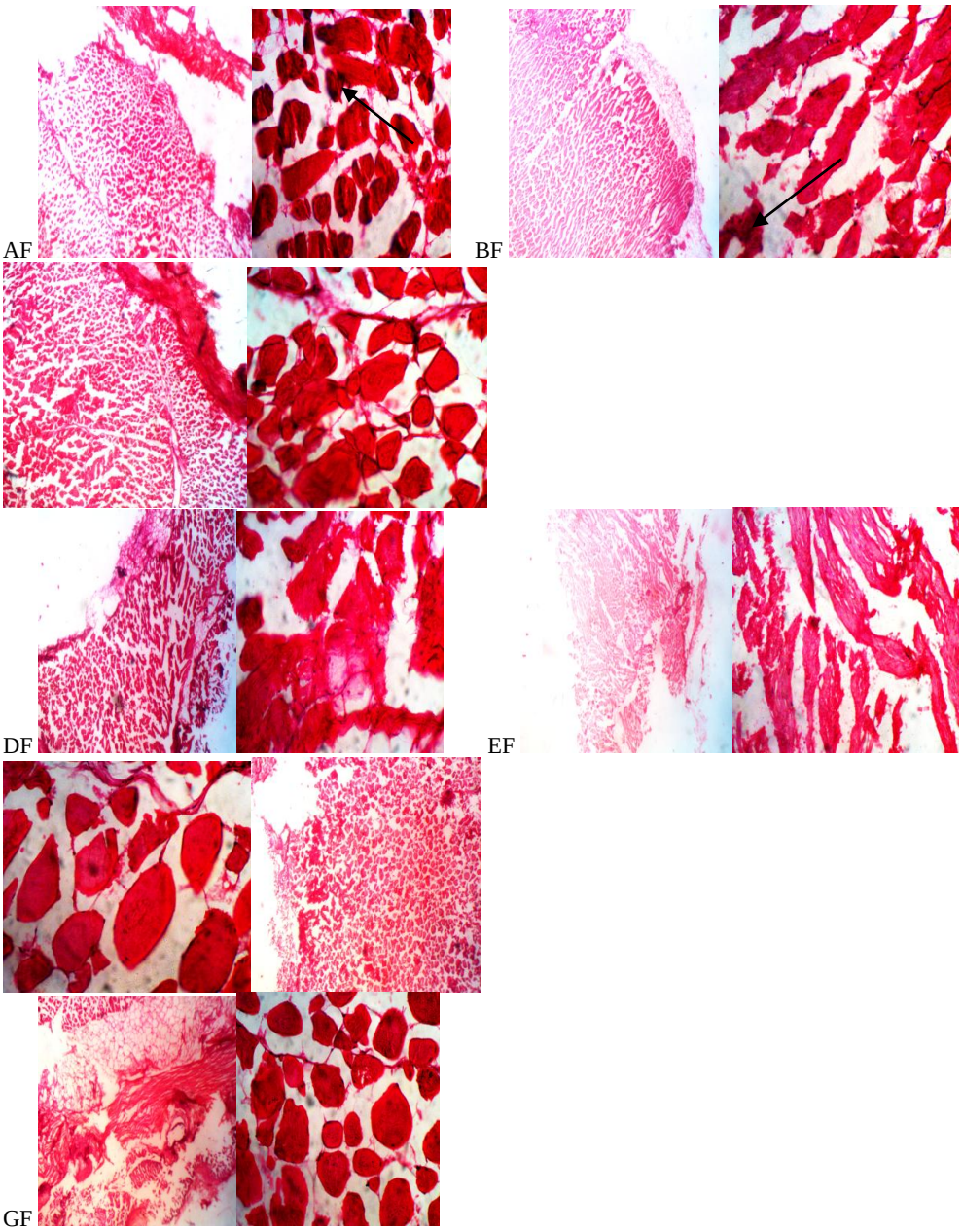

Plate 1 (AF- GF} = Transverse section of female muscle wound closure of *Clarias gariepinus* fed different inclusion levels of medicinal plants mixture. **(AF)** The control showed that there is accentuation of interstitial spaces and moderate myofibre atrophy. HE x100, 400, **(BF)** = muscle wound closure of *Clarias gariepinus* fed MPLM at 0.5% indicating there is accentuation of interstitial spaces and moderate myofibre atrophy. HE x100, 400, **(CF)** = muscle wound closure of *Clarias gariepinus* fed MPLM at 1.0% showing there is accentuation of interstitial spaces and moderate myofibre atrophy. HE x100, 400, **(DF)** = muscle wound closure of *Clarias gariepinus* fed MPLM at 1.5% indicate there is no observable lesion. HE x100, 400, **(EF)** = muscle wound closure of *Clarias gariepinus* fed MPLM at 2.0% indicate there is accentuation of interstitial spaces and moderate myofibre atrophy. HE x100, 400, **(FF)** = muscle wound closure of *Clarias gariepinus* fed MPLM at 2.5% indicate there is no observable lesion. HE x100, 400 **(GF)** = muscle wound closure of *Clarias gariepinus* fed MPLM at 3.0% indicate there is no observable lesion. HE x100, 400

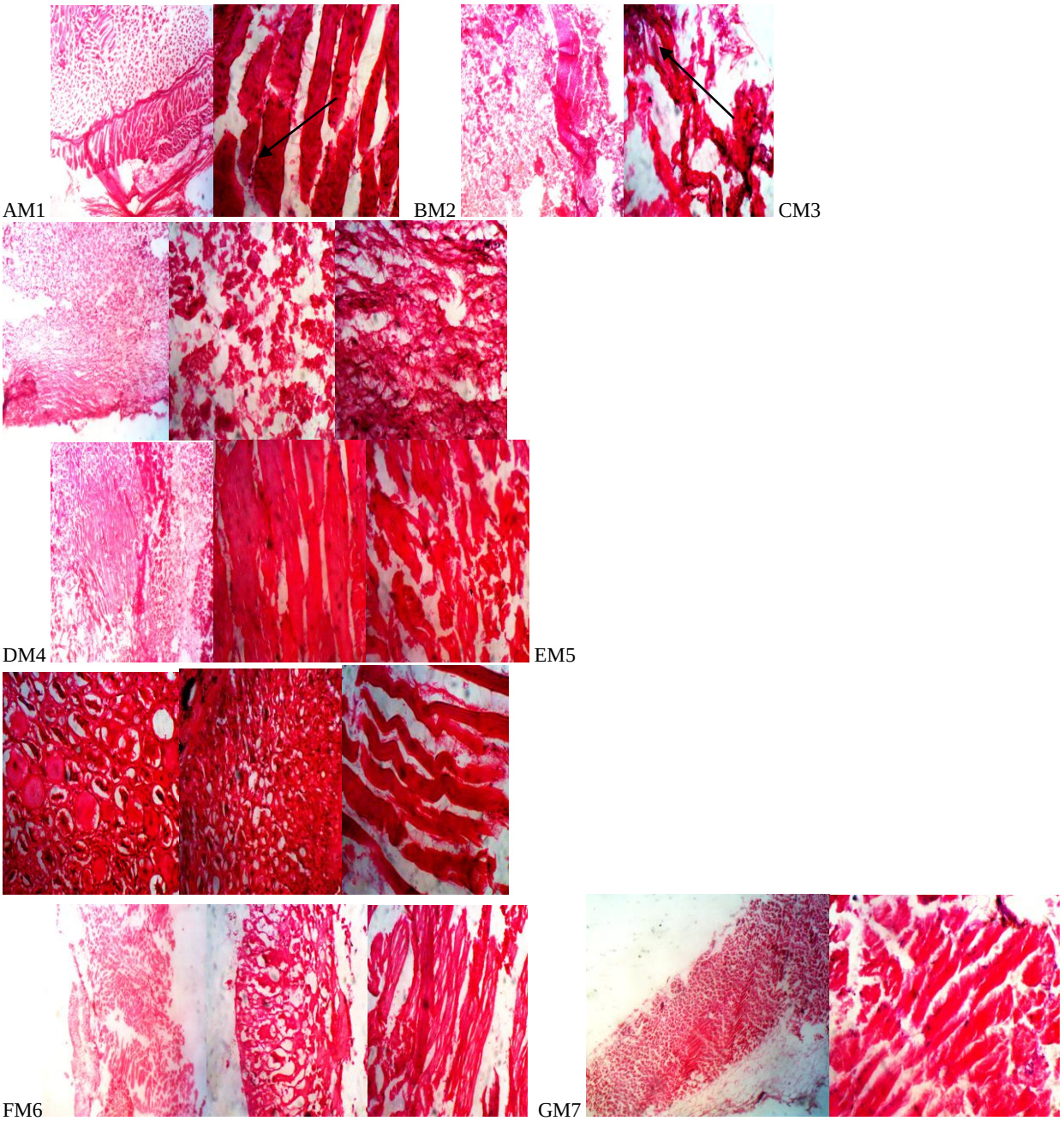

Plate 2 (AM- GM} = Transverse section of male muscle wound closure of *Clarias gariepinus* fed different inclusion levels of medicinal plants mixture **(AM)** = The control showed there is accentuation of interstitial spaces and moderate myofibre atrophy HE x100, 400, **(BM)** = muscle wound closure of *Clarias gariepinus* fed MPLM at 0.5% indicating there is accentuation of interstitial spaces and moderate myofibre atrophy. HE x100, 400, **(CM)** = muscle wound closure of *Clarias gariepinus* fed MPLM at 1.0% showing that there is no observable lesion. HE x100, 400, **(DM)** = muscle wound closure of *Clarias gariepinus* fed MPLM at 1.5% indicate there is no observable lesion. HE x100, 400, **(EM)** = muscle wound closure of *Clarias gariepinus* fed MPLM at 2.0% indicate there is no observable lesion. HE x100, 400, **(FM)** = muscle wound closure of *Clarias gariepinus* fed MPLM at 2.5% indicate there is no observable lesion. HE x100, 400, **(GM)** = muscle wound closure of *Clarias gariepinus* fed MPLM at 3.0% indicate there is no observable lesion. HE x100, 400.

Discussion

Phytochemicals have shown significant promise in the prevention and treatment of microbial infections and wounds (Vitale *et al.*, 2022). Anti-microbial, antioxidant, and wound healing phytochemicals encourage blood clotting, fight infection, and accelerate the healing of wounds (Vitale *et al.*, 2022). The results of the phytochemical analysis of this study confirmed the presence of saponins, tannins, flavonoids, steroids, protein,

alkaloids, glucosinolates and phenols. These results were similar to the report of Kumari *et al*., (2016), Garba *et al*., (2013) and Ujah *et al*., (2021), who observed the presence of saponins, tannins, flavonoids, steroids, protein, alkaloids, glucosinolates and phenols in fenugreek, garlic and scent leaf, respectively. These secondary metabolites, like the steroids, flavonoids and alkaloids, are all valuable medicinal plants which are widely used in many traditional cultures and are increasingly becoming popular in modern society as natural alternatives to synthetic medicines. The presence of alkaloids in garlic, stone breaker, fenugreek and scent leaves shows the potential of these medicinal plants to have analgesic, anti-inflammatory and adaptogenic effects, which help animals to develop resistance against disease and endurance against stress (Gupta, 1994). These could be possible as these medicinal plants contain some antibacterial activities. Saponins possess beneficial (cholesterol-lowering) properties and also exhibit structure-dependent biological activities (Osagie and Eka, 1998). Saponins cause a reduction of blood cholesterol by preventing its reabsorption (Prohp and Onoagbe, 2012), and in this study, saponins were present in garlic, fenugreek and scent leaves, but absent in stone breaker. Flavonoids are water-soluble polyphenolic molecules and therefore belong to the polyphenol family. Flavonoids have antioxidant activities as well as many health-promoting effects, such *as* anti-allergic, anti-cancer, antioxidant, anti-inflammatory, anti-thrombotic, and antiviral effects. These effects have been associated with the influence of flavonoids on arachidonic acid metabolism. Some flavonoid-containing plants are diuretics, anti-spasmodics, and others have antimicrobial properties (Trease and Evans, 2002). Getie *et al*., (2002) and Shetty *et al*., (2008) reported that flavonoids can promote excellent healing of wounds, likely by means of antimicrobial and antioxidant properties, by inhibiting lipid peroxidation, which leads to the prevention of cell damage and the increase in the viability of collagen fibrils. The flavonoids are acting on bacteria by inhibiting their protein synthesis (Hong-xi and Song, 2001) and in this study, flavonoids were present in garlic, stone breaker, fenugreek and scent leaves. Phenols present in plants have powerful medicinal effects as they can help to manage ulcers and cancer. Moreover, phenols and glycosides are both powerful secondary metabolites with significant antioxidant activities; these metabolites also contain vitamins, which play a vital role in animal health. Phenols are found in the scent leaves and fenugreek, but are absent in stone breaker and garlic. Deshmukh *et al*., (2009) and Lopes *et al*., (2005) reported that phenolics promote wound healing mainly due to their astringent, antimicrobial, and free radical scavenging properties. The faster wound healing of C. gariepinus may be a result of bioactive ingredients present in these plants.

Natural herbal remedies have now become fundamental for the management of skin disorders and the treatment of skin infections due to the side effects of modern medicine and lower prices for herbal products (Vitale *et al*., 2022). The results of this study show a general wound closure in the C. gariepinus-fed treated groups and the untreated group. At the 7th day post-wounding, gross investigations of male and female *C. gariepinus* wounds displayed more appropriate wound closure in the control and treatment groups, and reduction of wound size was notable. However, the treated groups had better wound healing and daily wound healing rate compared to the control. These observations were similar to the report of Bello *et al.*, (2013), who reported a higher percentage of healing and a daily healing rate of wound closure in *C gariepinus* fed onion bulb and walnut leaves supplemented diets. All wounds were completely re-epithelialized and covered with a new layer of epidermis in MPLM 5 for male and female *C. gariepinus* at 9 days, and at 14 days, there was no scar observed in the fish, while the control, MPLM 6 and MPLM 7 were completely healed, but with scar. Mucous cells were present on the surface of the new epidermis. Melanin pigments were also scattered throughout the thickness of the epidermis. This finding suggests that medicinal plants mixture supplemented diets may be more effective in dermal wound healing and studies on garlic, stone breaker, fenugreek and scent leaves have shown phytochemical such as flavonoids, phenols, and alkaloids that are known to promote wound healing process in animals due to their antimicrobial properties which appears to be major factors that responsible for wound contraction and increase the rate of epithelization and regeneration by promoting fibroblasts proliferation and/or collagen production. The results of the study show that males had faster wound closure in terms of percentage healing and daily healing rate due to the anabolic hormonal influence in the male *C. gariepinus* compared to the female *C. gariepinus*, which is also similar to the report of Bello *et al*., (2013).

Additionally, histopathological examination revealed that no visible abnormal structural changes were observed in the male and female *C. gariepinus* muscle wound closure, but slight observable changes were observed in the control group, MPLM 2, MPLM 3 and the control group and MPLM 2 for female and male *C. gariepinus*, respectively. In the control group of male and female *C. gariepinus*, weak regeneration of muscular tissue was observed, and the epidermis was thickened and irregular. Also, accentuation of interstitial spaces and moderate myofibre atrophy was observed. However, the inclusion of this plant mixture helps to reduce inflammation, decrease scar tissue size, and increase alignment and organisation of regenerated scar tissue, increasing the concentration of collagen and glycosaminoglycans compared to the control lesions. This suggests that the medicinal plants mixture had beneficial effects on *C. gariepinus* by stimulating collagen deposition, fibroblast proliferation, and angiogenesis and inhibiting the overproduction and accumulation of matrix proteins that cause hypertrophic scarring.

Conclusion

Medicinal plants have a great deal of therapeutic potential and could help wounds heal at different stages. These results suggest that *Trigonella foenum-graecum*, *Allium sativum*, *Ocimum gratissimum*, *and Phyllanthus niruri*, which are common in nature, may be promising antimicrobial and wound-healing agents for the treatment of wound healing as an alternative to synthetic drugs with high costs and side effects. Combination of these

Conflict of Interest

The authors declare that no conflict of interest was observed during the study.

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