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Potentials of *Tridax procumbens* Leaf Extract as Antimicrobial Agents for African catfish (*Heterobranchus bidorsalis*)

Ibrahim ADESHINA [1]
Yusuf Adetunji ADEWALE [2]
Mozeedah ABDULWAHAB [3]
Lateef Oloyede TIAMIYU [1]

[1] University of Ilorin, Department of Aquaculture and Fisheries, Ilorin, NİJERYA

[2] Deakin University, School of Environmental Life Sciences, Faculty of Built Engineering, Deakin, Avustralya

[3] University of Lagos, Department of Zoology, Lagos, NİJERYA

The increase in the demand for fish has led to intensive aquaculture practices which has also ushered in a manifestation and emergence of new fish diseases and reoccurrence of old ones. The advocacy for alternative therapy to drugs using natural and environmental friendly materials necessitated this study. The antimicrobial properties of *Tridax procumbens* extracts were tested against pathogenic fish bacteria (*Escherichia coli*, *Pseudomonas aeruginosa, Staphylococcus aereus, Aeromonashydrophilia,* and *Listeria monocytogenes*). Bacteria were cultured, minimum inhibitory concentration (MIC) and zone of inhibition (ZI) were carried out using standard methods. One hundred and eight (108) *Hetrobranchus bidorsalis* juveniles were fed *T. procumbens* based diets (40% crude protein) two times daily in a completely randomized designed for 54 days. Data obtained were analyzed using descriptive statistics and ANOVA at $\alpha_{0.05}$. The results shows that *T. procumbens* extract contained alkaloids, flavonoids, tannins, steroids and carptenoids while saponin and terpnoids were not detected in the extract. Effective MIC was 40mg/ml and ZI ranged from 12.85±0.39 to 14.20±0.74 mm. Pathogens were sensitive to the *T. procumbens* extract except for *L. monocyogenes.* The TVC and TCC were significantly reduced in the treated group. The results revealed *T. procumbens* leaves have antimicrobial properties which may had shown its potential utilization in aquaculture. It also indicated that *T. procumbens* have antimicrobial activity against tested organisms and could be used fish health management.

Key Words: *Antimicrobial activity, tridax procumbens, pathogens, fish*

Afrika kedibalığı (*Heterobranchus bidorsalis*) için Antimikrobiyal Ajanlar olarak *Tridax procumbens* Yaprak Özü Potansiyelleri

Balıklara olan talebin artması, yeni balık hastalıklarının ortaya çıkışı ve ortaya çıkışı ile eski balık yetiştiriciliğinin tekrar yaşanmasına neden olan yoğun akuakültür uygulamalarına da yol açmıştır. Doğal ve çevre dostu materyalleri kullanarak farmasötik ilaçlara alternatif terapi yapılmasının savunulması bu çalışmayı gerektirdi. Tridax procumbens özütlerinin antimikrobiyal özellikleri, patojenik balık bakterilerine (Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aereus, Aeromonashydrophilia ve Listeria monocytogenes) karşı test edilmiştir. Bakteriler kültürlendi, minimum inhibisyon konsantrasyonu (MIC) ve inhibisyon zonu (ZI) standart yöntemler kullanılarak gerçekleştirildi. Yüz sekiz (108) Hetrobranchus bidorsalis yavruya 54 gün süreyle tamamen randomize edilmiş olarak günde iki kez T. procumbens esaslı diyetler (% 40 ham protein) verilmiştir. Elde edilen veriler, betimsel istatistikler ve ANOVA kullanılarak α0.05'de analiz edilmiştir. Elde edilen sonuçlar, T. procumbens ekstraktının alkaloidler, flavonoidler, taninler, steroidler ve karptenoidler içerdiğini, ekstraktaki saponin ve terpoidlerin bulunmadığını göstermektedir. Etkili MİK 40 mg / ml idi ve ZI, 12.85 ± 0.39 ila 14.20 ± 0.74 mm arasında değişiyordu. Patojenler L. monocyogenes hariç T. procumbens ekstraktına duyarlıydı. TVC ve TCC, tedavi edilen grupta anlamlı olarak azaldı. Sonuçlar, T. procumbens yapraklarının, su ürünleri yetiştiriciliğinde potansiyel kullanımını göstermiş olabilecek antimikrobiyal özelliklere sahip olduğunu ortaya koymuştur. Ayrıca, T. procumbens'in test edilen organizmalara karşı antimikrobiyal etkinliğe sahip olduğunu ve balık sağlığı yönetiminde kullanılabileceğini belirtti.

***Anahtar* Kelimeler:** *Antimikrobiyal aktivite, tridax procumbens, patojenler, balıklar*

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Yazışma Adresi
Correspondence

Ibrahim ADESHINA
University of Ilorin,
Department of Aquaculture and Fisheries,
Ilorin – NİJERYA

adesina.i@unilorin.edu.ng

Introduction

Following the increase in the consumption of fish which majorly comes from aquaculture (1-2) the need for the safest animal source of protein especially fish has become obvious and cannot be over-emphasized. The demand has led to intensive aquaculture practices which is characterized by high yield and economic returns which has also ushered in a manifestation and emergence of new fish diseases and reoccurrence of old ones (3) and thus could resulted in great biological and economical losses. Since fish is capable of harbouring pathogens/diseases or serves as carrier, which could be transmitted to man through contact or consumption of infected fish it has become necessary to ensure food safety (4). Reported that import rejection of seafood and fish was approximately 15% out of total product rejection and thus bacteria pathogens became the major reason for product rejection (5).

In an attempt to combat these challenges, several methods have applied. One of the such methods is the use of chemotherapy. However, in recent times, strong recommendation against continuous and indiscriminate use of drugs have been encouraged due to residual side effects, environmental degeneration and noticeable development of antibiotic-resistant pathogens (6-7). Therefore, the need for alternative therapy using natural and environmental friendly materials cannot be over emphasized.

African catfish (*Heterobranchus bidorsalis*) is widely distributed fish in Africa, especially in Nigeria. *H. bidorsalis* belongs to the family Clariidae. It has ability survive in wide range of water and environmental parameters such as low dissolved oxygen levels which is usually harmful or lethal to other species. It has ability to consume varieties of conventional and non-conventional feed including kitchen waste (8-9). It has been cultured singly and/with other species in form of polyculture in Nigeria.

In Nigeria, use of medicinal plants in health management is very common locally, because traditional ways of treating diseases are well established and has contributed to the pharmaceutical industries. *Tridax procumbens* (family-Asteraceae) known as "*Ebueigba*" in Yoruba language is a perennial plant which available virtually in all seasons. It is a weak straggling herb about 12-24 cm long with few leaves 6-8 cm long and grows on available space including backyard in Nigeria. The leaves of this plant including other aerial parts except flowering tops is said to be useful in the treatment of inflammatory conditions, heal wound, anti-diabetic activity, anti-arthritic activity, preventing hair loss, diarrhoea and serve as insect repellent. Despite of the enormous advantage and benefit of *T. procumbens* it is majorly regarded as weed except in feeding of rabbit and therefore there is need to investigate the antimicrobial potential of *T. procumbens* in the control of fish diseases.

Oils from these plants were reportedly having inhibiting and bactericidal properties (10). It contains phenolic compounds, which possess antimicrobial activity. These compounds were classified as generally recognized as safe (GRAS) as such could be used in the control and management of bacterial in food (11-12). The oil from the plants have been reported to perform actively on many bacteria (*Staphylococcus, Salmonella, Listeria monocytogenes, Shigella,* and *Escherichia coli*) and fungi (*Trichophyton rubrum, T. mentagrophytes, Penicillium islandicum* and *Candida albicans)* respectively (13-14).

This study was therefore aimed at screening for the antimicrobial properties of *T. procumbens* against bacteria of fish with a possibility of determining the minimum inhibitory concentration by which the plant extract can be included in fish diets.

Material and Methods

Plant Collection and Identification: *Tridax procumbens* leaves were obtained from open space adjacent to Department of Aquaculture and Fisheries Management, University of Ibadan, Nigeria. The plant was authenticated at the herbarium of the Forestry Research Institute of Nigeria (FRIN), Ibadan and a voucher specimen was placed under FHI110831.

Preparation and Extraction of Plant Materials: The plant was rinsed with distilled water and aired at ambient temperature for 2 hours. Five (5) g of *T. procumbens* fresh leaves was weighed on a digital ScoutPro sensitive scale in the Department of Aquaculture and Fisheries Management, University of Ibadan, covered with sterilized cotton wool and transferred into Soxhlet apparatus as described by (11, 15). Briefly, about 170 mL of ethyl-acetate were poured in a spiral tube of the equipment. The extraction last for six hours until no further drop of extract in triplicates. The filtrate were concentrated on a rotary evaporator at 45°C for chemicals elimination, stripped into a sterile bottle and kept in a refrigerator until use.

Phytochemical Analysis: The extracts of fresh leaves of *T. procumbens* were both qualitatively and quantitatively examined for the presence of secondary metabolites using various analytical tests and reagents. The metabolites examined were Alkaloids (16-17), Flavonoids (17), Tannins (18), Saponins (18), Terpenoids (19), Steroids (20) and Carotenoids (21).

Media Preparation: MacConkey, Nutrient, Plate Count Agars, nutrient broth and peptone water were prepared according to Manufacturers' instruction (all agar were LAB-M products).

Microbial Analysis: The organs (liver, kidney, gill, intestine, flesh/muscle and spleen) were aseptically collected and weighed into sterile universal bottles while skin samples were collected using skin swab. The Samples were inserted into peptone water (0.1%) and allowed to release the available bacterial for a period of 2-3 hours. One mL was taken from each sample bottles and diluted in ten folds and subsequently serially diluted with dilution factor 10^{-4}. Two ml were taken from each sample and dispensed into two petri dishes (1 mL to each). The first dish received plate count agar (PCA) for total viable count (TVC) (LAB M, LAB149) while the second petri dish received MacConkey agar (LAB M, LAB002) for total coliform count (TCC) using the pure plate count method. The media were prepared according to manufacturers' instruction. Each dilution was overlaid with PCA and MacConkey respectively that has been cooled to 50^0C. At this temperature, agar is still in liquid form. The dishes were then gently swirled to mix the bacteria with the liquid agar (15). The mixtures were allowed to harden. When the mixture is hardened, the individual cells are fixed in place and incubated (Newlife Laboratory Incubator NL-9052-1) for 24 hours at 37 ºC to allow a distinguish colonies to form. The colonies formed were counted using Wincom Colony Counter (16W,

220V±10%, 50 Hz). The experiments were replicated three times. The total viable count and total coliform count were expressed in Log_{10}CFU/g (6, 21).

Antimicrobial Assay: Five pure bacteria isolates were obtained from Laboratory of Department of Microbiology, University of Ibadan, Nigeria. The isolates were: *Listeria monocytogenes, Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aereus and Aeromonashydrophilia*.

Detection of Antagonistic Activity: Agar well diffusion methods were adapted. From the extracts, 0.1 g were dissolved in 2 mL of Dimethyl Sulfoxide (DMSO) (22) and reconstituted to have 50, 40, 30, 20, 10, 5, 2.5, 1.25, 0.625 and 0.3125 mg/mL. A cork borer of 6 mm diameter was used to create wells on the plates. Each well was filled with the extracts at the working concentration sterilized droppers and incubated at 37 ºC for 24 to 48 hours. The zones of inhibition were measured and minimum inhibitory concentrations were determined.

Feeding Trial:

Feed Preparation: The best two concentrations that produced better zones of inhibition were incorporated in to feed formulated at 40% crude protein and fed to fish for 54 days. Organs were asceptically collected and examined for TCC and TVC. The feed ingredients (Table 1) were pelleted (2.0 mm) and packed in a polythene bags and store in a cool dry place until use with a well label on it.

Table 1. Gross composition of experimental diets

	Control	*Tridax procumbens*	
		T1	T2
Fish meal	30.83	30.83	30.83
Soybean	30.83	30.83	30.83
Maize	33.84	33.79	32.80
Starch	1.00	1.00	1.00
VegeTable oil	1.50	1.50	1.50
Premixes*	2.00	2.00	2.00
Extract	0.00	0.05	0.04
Total	100.00	100.00	100.00

* Premixes= HI-MIX®AQUA (Fish) each one kilogram (1 kg) contains; vitamin A 4.000.000 international Unit (IU); vitamin D_3 800.000 IU; vitamin E 40.000 IU; vitamin K_3 1.600 mg; vitamin B_1 4.000 mg; vitamin B_2 3.000 mg; vitamin B_6 3.800 mg; vitamin B_{12} 3 mcg; nicotinic acid 18.000 mg; pantothenic acid 8.000 mg; folic acid 800 mg; biotin 100 mcg; choline chloride 120.000 mg; iron 8.000 mg; copper 800 mg; manganese 6.000 mg; zinc 20.000 mg; iodine 400 mg; selenium 40 mg; vitamin C C (coated) 60.000 mg; inositol 10.000 mg; colbat 150 mg; lysine 10.000 mg; methionine 10.000 mg; antioxidant 25.000 mg.

Experimental Procedure: A total number of one hundred and eight (180) *Heterobranchus bidorsalis* juveniles (12.20±1.12 g) were used for the experiment. The fish were acclimatized for 2 weeks in plastic tanks before the experiment. Fish were weighed and distributed in to nine rectangular plastic tanks (60 x 38 x 27 cm) tanks in a completely randomized design. Each tank contained twenty fish and water was replaced on three days interval. The experiment had three treatments containing 0.0% (control), 0.05% (T1) and 0.04% (T2) of *T. procumbens* extracts in triplicates. The fish were fed at 3% body weight daily (1.5% was given in the morning by 8:00 am and 1.5% in the evening by 5:00 pm) throughout the experimentation period of 54 days. Water quality parameters were measured while the experiment lasted for fifty-four days. The protocol of this study was subjected to ethical consideration and received approval from University of Ibadan Animal Care Use and Research Committee (UI-ACUREC; U.I.ACUREC/IA/16/0005-UI-ACUREC/App/03/2017/008)

Statistical Analysis: The data obtained were subjected to descriptive statistics and one-way analysis of variance (ANOVA) at $\alpha_{0.05}$ using SPSS IBM version 20. Microbial load was expressed as log_{10} and reported as mean. The means were separated using Duncan multiple range test.

Results

Secondary metabolites observed were alkaloids (156.67±3.02 mg/100 g), flavonoids (130.12±5.29 mg/100 g), tannins (107.21±4.17 mg/100 g), steroids (112.306±8.39 mg/100 g) and carptenoids (84.77±3.85 mg/100 g) while saponin (23.08±8.01 mg/100 g) and terpnoids (18.02±1.61 mg/100 g) were detected in minute quantities in the extract (Table 2) of the *T. procumbens*. It was observed that most of the parameters examined were present and significantly different (P=0.02).

Table 2. Screening of phytochemicals of extract of *T. procumbens*

Parameter	Observation	Quantification (mg/100g)
Alkaloids	+	156.67±3.02[a]
Flavonoids	+	130.12±5.29[a]
Tannins	+	107.21±4.17[b]
Saponin	-	23.08±8.01[d]
Terpnoids	-	18.02±1.61[d]
Steroids	+	112.306±8.39[b]
Carotenoids	+	84.77±3.85[c]
P-vaue		0.027

+ = present; - = absent; Means with different superscripts in the same row are statistically significant different.

Extract of *T. procumbens* at concentrations of 0.00 to 2.5 mg/mL did not inhibit the tested pathogens. However, concentrations from 10 mg/mL to 50 mg/mL showed inhibition of the organisms except *L. monocytogenes* which did not show any inhibition. No growth of *E. coli* and *A. hydrophilia* were observed at 20 mg/mL while *P. aeruginosa* and *S. aereus*did not grow at 10 mg/mL and 5 mg/mL respectively and thus 20 mg/mL is minimum inhibitory concentration (Table 3).

The antagonistic performance of *T. procumbens* extract against the tested pathogens revealved that highest zone of inhibition was observed in 50 mg/mL

extract tested on *S. aereus* (16.23±1.33 mm) while the least was recorded in control (0.00±0.00 mm) at 0.00 mg/mL concentration. More so, there were progressive increase in the zones of inhibition observed in tested organisms with increase in the concentration of the extract except in *L. monocytogenes* which did not show any clear zone of inhibition (Table 4). There were significant differences among the extracts against the pathogens (P<0.05).

Results of microbial load investigations indicated that both total viable bacteria count (TVC) and total coliform count (TCC) were higher in fish tissues before feed trial than after the feed trial. After the feeding trial, the highest TCC and TVC were recorded in fish treated control diet and the least in group fed fortified diet. Skin had highest TCC and TVV while the least TVC and TCC were recorded in muscle. There were significant differences in the TVV and TCC among the treatments across the organs (Table 5).

The mean dissolved oxygen was 6.80±1.10 mg/L; temperature was 25.15±2.66 and pH was 7.28±0.81 (Table 6).

Table 3. Minimum Inhibitory Concentration of ethyl-acetatic extract of *Tridax procumbens*

Isolates	Concentrations (mg/mL)										
	50	40	30	20	10	5	2.5	1.25	0.625	0.3125	0.00
Escherichia coli	-	-	-	-	+	+	+	+	+	++	++
Pseudomonas aeruginosa	-	-	-	-	-	+	+	+	+	++	++
Listeria monocytogenes	+	+	+	++	++	++	++	++	++	++	++
Staphylococcus aereus	-	-	-	-	-	-	+	+	+	++	++
Aeromonas hydrophilia	-	-	-	-	+	+	+	+	+	++	++

++ = high growth observed; + = sparsely growth observed; - = no growth observed

Table 4. Zone of inhibition of ethyl-acetatic extract of *Tridax procumbens*

Isolates	Zone of inhibition (mm)							P-value
	50	40	30	20	10	5	0.00	
E. coli	14.01 ± 1.12^{aB}	13.22 ± 1.31^{aB}	7.69 ± 0.53^{bA}	7.02 ± 0.82^{bA}	5.31 ± 0.23^{cA}	4.65 ± 1.10^{cA}	0.00 ± 0.00^{d}	0.015
P. aeruginosa	14.37 ± 0.55^{aB}	13.00 ± 1.23^{aB}	8.34 ± 1.01^{bA}	5.45 ± 0.43^{bB}	4.02 ± 0.02^{bA}	4.01 ± 0.58^{bB}	0.00 ± 0.00^{c}	0.006
L. monocytogenes	0.00 ± 0.00^{aC}	0.00 ± 0.00^{aC}	0.00 ± 0.00^{aC}	0.00 ± 0.00^{aC}	0.00 ± 0.00^{aB}	0.00 ± 0.00^{aC}	0.00 ± 0.00^{a}	-
S. aereus	16.23 ± 1.33^{aA}	14.20 ± 0.74^{aA}	8.27 ± 1.20^{bA}	4.22 ± 0.03^{bB}	4.16 ± 0.02^{bA}	3.76 ± 0.00^{bB}	0.00 ± 0.00^{c}	0.032
A. hydrophilia	15.74 ± 0.54^{aA}	12.85 ± 0.39^{bA}	5.23 ± 0.11^{cB}	5.02 ± 0.02^{cB}	4.53 ± 0.12^{cA}	2.45 ± 0.04^{cB}	0.00 ± 0.00^{d}	0.004
P-value	0.002	0.005	0.024	0.016	0.019	0.001	-	

Values are presented as mean ± standard deviation of triplicates. Means with different small letter superscripts in the same row are statistically significant different; means with different capitall letter superscripts in the same column are statistically significant different

Table 5. Microbial load of fish tissues

Tissues	Microbial load (log_{10}CFU/g)				
	Before	After			
		T1	T2	Control (0.00)	P-value
			Total coliform count (TCC)		
Liver	5.63 ± 0.21^{aA}	2.04 ± 0.02^{bB}	2.66 ± 0.03^{bB}	5.54 ± 0.11^{aB}	0.021
Kidney	5.23 ± 0.45^{aA}	2.73 ± 1.02^{bB}	2.80 ± 1.00^{bB}	5.41 ± 0.37^{aB}	0.010
Intestine	5.05 ± 0.32^{aA}	2.35 ± 0.23^{bB}	3.01 ± 0.64^{bB}	5.23 ± 0.20^{aB}	0.035
Muscle	4.20 ± 0.18^{aA}	2.00 ± 0.11^{bB}	2.78 ± 0.08^{cB}	4.31 ± 0.20^{aB}	0.012
Spleen	4.45 ± 0.29^{aA}	2.39 ± 1.22^{bB}	3.05 ± 0.36^{cB}	4.52 ± 0.32^{aB}	0.004
Skin	6.52 ± 0.53^{aA}	3.65 ± 0.21^{bA}	4.02 ± 0.06^{bA}	7.22 ± 0.30^{aA}	0.041
Gill	6.21 ± 0.37^{aA}	3.53 ± 1.50^{bA}	3.57 ± 0.84^{bA}	6.37 ± 1.02^{aA}	0.018
P-value	0.854	0.033	0.027	0.001	
			Total viable count (TVC)		
Liver	6.22 ± 0.40^{aA}	3.03 ± 0.02^{bB}	2.90 ± 0.88^{bB}	6.43 ± 0.35^{aA}	0.003
Kidney	6.36 ± 0.71^{aA}	3.42 ± 0.33^{bB}	3.50 ± 0.12^{bB}	6.52 ± 0.34^{aA}	0.014
Intestine	6.13 ± 0.09^{aA}	2.77 ± 0.20^{bB}	$2.77.0.37^{bB}$	6.04 ± 0.12^{aB}	0.006
Muscle	5.36 ± 0.34^{aA}	2.54 ± 048^{bB}	3.13 ± 0.26^{bB}	5.34 ± 0.13^{aB}	0.012
Spleen	5.72 ± 0.30^{aA}	2.70 ± 0.24^{bB}	3.25 ± 0.31^{bB}	5.69 ± 0.26^{aB}	0.007
Skin	7.58 ± 0.92^{aA}	4.67 ± 0.52^{bA}	5.22 ± 0.39^{bA}	8.01 ± 0.43^{aA}	0.018
Gill	7.21 ± 0.45^{aA}	4.03 ± 0.43^{bA}	4.50 ± 0.25^{bA}	7.53 ± 0.70^{aA}	0.035
P-value	0.721	0.043	0.006	0.022	

Values are presented as mean ± standard deviation of triplicates. Means with different small letter superscripts in the same row are statistically significant different; means with different capitall letter superscripts in the same column are statistically significant differen

Table 6. Water quality parameter recorded during rearing of *Heretrobranchus bidorsalis* fry for 54 day

Parameter	Range	Mean
Dissolved oxygen (mg/L)	5.7 – 7.9	6.80±1.10
Temperature (ºC)	22.45 – 27.77	25.15±2.66
pH	7.24 – 7.33	7.28±0.81

Discussion

Phytochemicals in the plants has been used as an indices in predicting the antimicrobial activities of the plants. The leaves had as alkaloids, flavonoids, tannin, steroids and carotenoids which are considered relatively abundance. The result of this study is in agreement with the work of (23-25) who reported the presence of flavonoids, tannin, alkaloids, saponin in the methanolic extract of *T. procumbens.* The continuous and indiscriminate use of synthetic drugs has ushered in drug resistance pathogens, residual effects among others. The trend of minimum inhibitory concentration observed in this study follows the trend reported by (26-27).

The presence of flavonoids, an agent that have counter-action against free radicals which gesticulating disorder like inflammation and hepatotoxins could be responsible for inhibition and reduction of bacteria load in fish tissues. Tannin is refers to as antioxidant and antimicrobial agents (28) and thus its presence could be associated with the effective performance of *T. procumbens* in the diet of fish and in the invitro study. The lower MIC observed in *P. aeruginosa* and *S. aereus* revealed that *T. procumbens* at lower concentrations would be adequate to control the pathogens in fish farming. Similar observations were made by (6, 29) who reported 500µg/ml of onion bulb and walnut leaves against *P. aeruginosa*.

The widest zone of inhibition obtained against *S. aereus* showed that the pathogen is highly sensitive to the extract. According to the (25) acceptable zone of inhibition of effective plant extract should be 13 mm and above. Therefore, *T. procumbens* extract at 40 and 50 mg/mL had zones of inhibitions within the recommended values. The zones of inhibition obtained in the study for *E. coli, S. aereus, P. aeruginosa* and *A. hydrophila* corroborate with the findings (6, 30) who reported 9 to 13.5 mm zone of inhibition of plants extract against pathogenic bacteria including the tested organism of this study. The factors responsible for the higher zones of inhibition in this study may be attributed to antibacterial properties in the plants such as tannin.

The result of this study shows that there were amazing reduction in the TVC and TCC in groups treated *T. procumbens* extract based diet. According to international Commission on the Microbiological Specification of Foods (31) acceptable level of bacteria load in fish should be between $10^2 – 10^7$per gram or cm^2 equivalent to about 5.70 Log10cfu/cm^2 for skin and in the same vein (32) recommended 10^5 per gram for other part of fish as acceptable level. Therefore, the result obtained in this study before fed extract based diet and control after the trial had value higher than the recommended values. However, fish fed extract based diets had values in both TVC and TCC lower than the recommended values which is significantly different when compared to control. The result of this study is in agreement with the findings of (3, 6). Furthermore, it was noticed that skin and gill had higher bacteria load than other tissues which may be connected to the fact that they are external organs. These external organs usually have direct and frequent contact with the environment and thus lead increase bacterial load on the sites.

The results revealed *T. procumben* sleaf have antimicrobial properties which may had shown it potential utilization in aquaculture. It also indicated that *T. procumbens* is an effective plants and can be used to control many common pathogenic bacteria such as *Escherichia coli*, *Pseudomonas aeruginosa, Staphylococcus aereus* and *Aeromonas hydrophilia* with minimum inhibitory concentration is 40 mg/mL without deleterious effect on the water.

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