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RESEARCH PAPER

Preservative Effect of *Vernonia amygdalina* Extract on Microbial Activity, Proximate Composition and Sensory Evaluation of Smoked-dried African Catfish *Clarias gariepinus* (Burchell, 1822)

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Abstract

The effect of *Vernonia amygdalina* extract on microbial activity, proximate composition and sensory evaluation of smoked-dried *Clarias gariepinus* stored at 32.0±2°C was determined over the period of 21 days. The experimental treatments were the control, 0.5%, 1%, 1.5% (w/v) *Vernonia amygdalina* extract solutions. 52 fish of average mean weight of 30.98±1.32g were degutted, washed and randomly assigned to the treatments and soaked in the extract solutions for 30 minutes and later smoked-dried for 12 hours. Six bacterial species namely, *Bacillus subtilis, Corynebacteria sp, Proteus mirabilis, Streptococcus faecalis, Staphylococcus albus* and *Staphylococcus aureus* were observed in the study. There was no increase in the microbial loads in all the treatments except in the control which increased from 27×10^3 to 40×10^3 cfu. Highest crude protein content (48.08±0.82%) was recorded in the control while the least value (39.67±0.04%) was obtained in the fish treated with 0.5% concentration of the extract. There was no significant difference ($P>0.05$) in the sensory evaluation among all the treatments and the control. This study reveals that *V. amygdalina* extract can be used as preservative in protecting smoked-dried *C. gariepinus* from bacterial spoilage, thus limiting economic loss and possible health risk to consumers.

Introduction

Fish is highly perishable because it provides favourable medium for the growth of microorganisms after death (Al-Ghabshi *et al*., 2012). An estimate of 40% postharvest losses of total fish landings have been reported in Nigeria. Fish spoilage is influenced to a large extent by high ambient temperatures, considerable distances from landing sites to the points of utilization and inadequate infrastructure for postharvest processing, handing and preservation techniques. Thus, it is imperative to process and preserve some of the fish caught in the period of abundance, so as to ensure an all year round supply. This will invariably reduce postharvest losses, increase the shelf-life of fish, and guarantee a sustainable supply of fish during off season with concomitant increase in the profit of the fishermen. Proper preservation starts from the moment fish is harvested until it reaches the consumer's table (Oluborode *et al*., 2010).

Over the years, there has been a constant increase in the search of an alternative and efficient compound for fish preservation aimed at a partial or total replacement of antimicrobial chemical additives (Bate and Bendall, 2010). However, preservation are chemical agents intentionally added to food product to prevent or inhibit spoilage caused by bacteria, enzymes, mold, yeast and chemical changes, particularly borrowing reactions in discolorations and off flavours. The preservations are classified into categories; they are natural and synthetic preservation. The use of synthetic anti-oxidants and synthetic antibiotics has been widely used in most

smoked-fish industries to retard rancidity of oil in fish and thus preventing the entry of pathogens (Iheagwara *et al*., 2019). However, the use of these synthetic anti-oxidants has been banned in many countries because of their negative effects on the enzymes of the liver and lungs and if use regularly, will only create a defensive mechanism for the pathogens (Deresse, 2010). This necessitated the need to use of natural anti-oxidants and antimicrobial agents such as leave extract to improve the shelf life and prevent rancidity in smoke-dried fish**.**

Vernonia amygdalina commonly called bitter leaf is the most widely cultivated species of the genus *Vernonia* which has about 1,000 species of shrubs (Abdullahi *et al*., 2019). It belongs to the family Astaraceae. It is vegetatively cultivated by stem cutting and popular in most of West African countries including Nigeria, Cameroon, Gabon and Congo Democratic Republic. It was named after an English Botanist William Vernon. It is also referred to as ironweed. Bitter leaf is called Omjunso in East Africa especially Tanzania, Onugbo in Igbo and Orugbo among the Itsekiri and Urhobo tribes, Ewuro (Yoruba), Etidot (Ibibio), Ityuna (Tiv), Oriwo (Edo), Shiwaka (Hausa). The leaves are eaten, after crushing and washing thoroughly to remove the bitterness. All parts of the plant are pharmacologically useful. Both the roots and leaves are used in phytomedicine to treat fever, hiccups, kidney disease and stomach discomfort, among others (Abdullahi *et al*., 2019). Antihelmitic and antimalarial properties (Abosi *et al*., 2003) as well as antitumorigenic properties (Izerbigie *et al*., 2004), have also been reported for extracts from the plant. The aqueous extract of the leaves has been found to inhibit the growth of the gram +ve bacterium *Staphylococcus aureus* and gram –ve bacterium *Escherichia coli* (Oboh and Masodje, 2009). There has not been fully documented report on the preservative effect of *V. amygdalina* extract on microbial activity, proximate composition and sensory evaluation of smoked-dried *C. gariepinus.* Therefore, this research was designed to assess the possibility of improving the shelf life of smoked-dried *C. gariepinus* using *V. amygdalina* extract as preservative and its effect on proximate composition and sensory evaluation.

Materials and Methods

Study Area

The study was conducted in fish processing unit of the Department of Fisheries, Faculty of Agriculture, University of Maiduguri, Borno State, Nigeria. The department is located at latitude 11^{o} 8'N and longitude 13^{o} 3'E and the state has two distinct seasons, that is rainy season with annual rain fall of about 600mm from July to October and a hot dry season from March to July. The dry season is preceded by a period of harmattan (November to February) with very low temperature (Abdullahi *et al*., 2019).

Procurement of the Bitter Leaf

Fresh leaves of *V. amygdalina* were collected from Shoakari village, Jere local government Maiduguri, Borno state, Nigeria.

Procurement of Fish Samples

A total of 7000g of fresh African catfish (*Clarias gariepinus*) was procured from Gamboru fish market in Maiduguri Metropolis Council, Borno State which ranges in length from 23.95 – 26.65cm. The collection was done in the early morning in cold flask and transported to the fish processing unit where processing and smoking of the fish were carried out. The fish were degutted, weighted and thoroughly washed with clean water.

Preparation of *Vernonia amygdalina* Extract

The fresh leaves were air dried under shade for 48 hours and then grounded into fine powder using pestle and mortar, a solution was prepared by adding separately specific quantity (5g, 10g and 15g) of the bitter leave powder extracts to 1000ml of distilled water to form three treatments of 0.5%, 1% and 1.5% concentration respectively and were allowed to stay for 24 hours. No extract was added to the control treatment.

Experimental Design

The fish were randomly assigned to four experimental treatments. The treatments were divided based on the concentration of the extract in the solution to 0%, 0.5%, 1% and 1.5%. Each treatment was triplicated with total weight of 500g fish. The fish were soaked into the aqueous solution of the bitter leaf extract for 30 minutes. Thereafter, the fish were placed on wire mesh and allow to drain under shed. Light was set for the smoking kiln to glow for ten minutes. Then the fish were arranged based on their treatments and replications in the smoking kiln consisting of three racks and hard wood was used for ignition. The smoking process took 12 hours and the fish were weight at the first 30min and after each 1 hour for all the smoking period until a minimum weight was obtained and the temperature was regulated between 45-85^{o}C (Abdullahi *et al.*, 2019). After the smoking the smoked-dried fish were allowed to cool and packed in different cartons based on their treatments and then transferred to a cool dry place save from any contamination in the laboratory. Samples were taken for microbial analysis at seven days' interval for a period of 21 days.

Total Bacterial Count

The total bacterial count was carried out following the method described by Abdullahi *et al.* (2019) tenth fold of serial dilution with normal saline was used. 9ml each of the normal saline was dispensed in a sterilized test

tube and 1gram of the sample was weighed and transferred into a sterile universal bottle and 9ml of normal saline was added and shake well. Then 1ml was taken using sterile tests to make 10-fold serial dilution, then 0.1ml was transferred from the 3rd tube to a sterile dried nutrient agar and spread, it was later incubated at 37^{o}C for 24 hours. The colony was counted using colony counter and result was recorded and presented as colony forming unit (cfu).

Identification of Microorganism

The isolates were identified by conventional methods starting with grams staining briefly, using a sterile wire loop a drop of distilled water was put on the centre of grease-free slide and a portion of colony was picked and emulsified into drop of sample and allow to air dry before fixing to gram stain, crystal violet was then applied for 3 minutes (Abdullahi *et al*., 2019). It was then replaced with a gram's iodine for one munities, priori to rinsing with water and application of 95% alcohol until no colour appeared on the flow. Slides were then rinsed with water and safranin was applied for 1-2 minutes. This was followed by rinsing and air drying and then observed microscopically under x 100 emersion oil objective (Abdullahi *et al*., 2019).

Proximate Composition Determination

The proximate composition of the fish samples, before and after smoking were determined according to Association of Official Analytical Chemist (AOAC, 2019). The percentage of moisture content, crude protein, crude fibre, ether extract or lipid, ash, carbohydrate or nitrogen free extract (NFE) were determined.

Moisture Content

This was done based on the difference between the net weight and the weight after drying to a constant (at 100^{o}C) for 24 hours. Empty clean beakers, one for each sample were dried in a hot air oven at 100^{o}C for 30 minutes. Each beaker was then weighed (W1). Five grams of the sample were placed in the beaker and then reweighed (W2). The beaker with the sample was then dried in an oven at 60^{o}C for 72 hours, for a constant weight to be attained, and then transferred to a desiccator to cool. The beaker was quickly weighed with minimum exposure to the atmosphere (W3). The loss in weight of the sample during the drying represented the moisture content, while the dry matter was the weight obtained after drying the sample.

$$\% \text{ Moisture} = \frac{W2 - W3}{W2 - W1} \times 100$$

Where:
W1 = Weight of empty crucible
W2 = Weight of known amount of sample (fresh) + crucible
W3 = Weight of oven-dried sample

Crude Protein

The crude protein content was analyzed using Kjeldahl tablets and 1gram of the samples was weighed into a digestion tube and Kjeldahl tablets were added. 10ml of concentrated Sulphuric acid (H_2SO_4) was added onto the tube and digested at 420^{o}C, for 3 to 5 hours. After cooling, 80ml of distilled water was added into digested solution. About 50ml of 40% Caustic Soda (NaOH) was added to 50ml of the digest and then placed on the heating section of the distillation chamber. 30ml of 4% boric acid, bromocreasol green and methyl red (as an indicator) were put into a conical flask and placed underneath the distillation chamber for collection of ammonia, the solution changed from pink to green colour. About 0.1 normal solution of hydrochloric acid (HCL) was weighed into a burette. The conical flask containing the solution was titrated until the colour changed from green to pink. The burette reading was taken. The crude protein was calculated using the formula:

$$\% \text{ CP} = \frac{(A - B) \times N \times F \times 6.25}{\text{Weight of samples}} \times 100$$

Where:
A = Volume of acid used for titrating the samples (in ml)
B = Volume of acid used for titrating blank sample (0) in ml
N = Normality of acid used for titration
F = Factor (14.007)
6.25 = Constant

Crude Fibre

Crude fibre was determined by weighing 2g of the samples which was placed in a round or flat bottom flask and 50ml tri-chloro-acid reagent (TCA) added, the mixture was boiled and refluxed for 40minutes. Filter paper was used to filter the residues. The residue obtained was washed four times with hot water and once with petroleum ether or petroleum spirit. Then the filter paper and the sample were folded together and dried at 30^{o}C to 60^{o}C in an oven for 24hours, weighed, ashed at 650^{o}C then cooled and weighed.

$$\% \text{ CF} = \frac{\text{Difference in weight}}{\text{Weight sample on dry matter basis}}$$

Ether Extract

The ether extract was determined by using Soxhlet apparatus, 1g of the sample was weighed into filter paper and 100ml of petroleum ether was measured and

added into a flat bottom flask, this solution was heated at 45℃ for 2hours. The collecting flask was removed, cooled in desiccator for 15 minutes and the percentage (%) fat in the sample determined using the formula:

$$\% \text{ Fat} = \frac{\text{Weight of flask + fat after extraction}}{\text{Weight of samples}} \times 100$$

Ash

The ash content was determined by weighing 1gram of the samples into crucible and dried at 105℃ for 24hours, then cooled in the desiccator for 15 minutes and weighed. This placed in a muffle furnace for 2 – 3 hours at 600 to 650℃, then cooled in a desiccator for 15minutes and weighed.

$$\% \text{ Ash} = \frac{\text{Loss in weight}}{\text{Initial weight}} \times 100$$

Nitrogen Free Extract (NFE)

Percentage free extract was determined by computing:

$$\% \text{ NFE} = 100 - (\% \text{ CP} + \% \text{ CF} + \% \text{ EE} + \% \text{ Ash})$$

Where:
CP = Crude Protein
CF = Crude Fibre
EE = Ether Extract

Sensory Evaluation

The smoked-dried fish were subjected to sensory evaluation using a 10-man panel from the Department of Fisheries. The fish samples (treatment groups) were assessed in terms of organoleptic characteristics such as; colour, appearance, flavour, taste and general acceptability based on a 7-point hedonic scale (where; 7=excellent; 6=very good; 5=good; 4=fair; 3=poor; 2=very poor; 1=extremely poor) as described Mohammed *et al.* (2023).

Statistical Analysis

All the data obtained were subjected to the one-way analysis of variance (ANOVA) and a significance test for difference among sample variance was done using the Least Significance Difference (LSD) in the mean comparison of means at P≤0.05 level of significance with aid of Statistix (version 9.0).

Results

Percentage Weight Loss of *Clarias gariepinus* After Smoking Period for Twelve Hours

Table 1 shows the percentage weight loss of *C. gariepinus* after smoking for twelve hours with different concentrations of *Vernonia amygdalina* extract at temperature of 85°C for the first hour and the remaining eleven hours maintained the temperature of heat at 45-85°C.

Changes in Microbial Loads (populations cfu/g) of Smoked-dried *C. gariepinus* Stored at Room Temperature

Changes in microbial loads of smoked-dried *C. gariepinus* treated with different concentration of *Vernonia amygdalina* extract during storage at room temperature for the period of twenty-one (21) days is presented in Table 2. After one week (7) of storage, the population of bacterial loads decreased in all the treated samples except in control which increased from (27×10^3) to (37×10^3). After (21) days of storage, decreasing in microbial loads was observed in all levels of concentration except for the control with 0% extract concentration which increased from (38×10^3) to (40×10^3). Smoked-dried *C. gariepinus* treated with 1.5%

Table 1. Percentage weight loss of *Clarias gariepinus* after smoking period for twelve hours with different concentrations of *Vernonia amygdalina* extract

Concentrations (%)	Initial weight (g)	Final weight (g)	Weight loss (g)	% Weight loss
0.0	500	170	330	66
0.5	500	175	325	65
1.0	500	180	320	64
1.5	500	200	300	60

Table 2. Changes in Microbial loads (populations cfu/g) of smoked-dried *Clarias gariepinus* during storage at room temperature for twenty-one (21) days

Extract Concentration level (%)	1 day	7 days	14 days	21 days
0.0	27×10^3	37×10^3	38×10^3	40×10^3
0.5	25×10^3	23×10^3	16×10^3	12×10^3
1.0	26×10^3	20×10^3	13×10^3	10×10^3
1.5	25×10^3	24×10^3	9×10^3	7×10^3

solution of the bitter leaf extract was recorded the least microbial loads (7×10^3) after the storage period.

Bacteria Isolated in *Clarias gariepinus* Smoked with Different Concentration of *Vernonia amygdalina* Extract

The amount and percentage of bacteria isolated in *Clarias gariepinus* smoked with different concentration of *V. amygdalina* extract are presented in Table 3 and Figure 1, respectively. Five bacteria species were isolated, *Bacillus subtilis, Corynebacteria species, Streptococcus faecolis, Staphylococcus albus* and *Staphylococcus aureus*. Highest percentage of bacteria isolated was recorded in sample smoked with 0% and 0.5% concentration of the bitter leaf extract with same value of 28% and lowest percentage was recorded in sample treated with 1.0% and 1.5% of the extract with same value of 22%.

Proximate Composition of Smoked-dried *Clarias gariepinus* Treated with Different Concentrations of *Vernonia amygdalina* Extract

Proximate composition of smoked-dried *C. gariepinus* treated with different concentrations (0.0, 0.5, 1.0 and 1.5%) of *V. amygdalina* extract is presented in Table 4. Highest value of moisture content 11.35% was recorded

Table 3. Bacterial isolation and identification in *Clarias gariepinus* smoked with *Vernonia amygdalina* extract at different concentration level for 21 days

Bacterial isolation	Bitter leaf extract Concentration (%)	No. of species	Percentage (%)
Bacillus subtilis *Corynebacteria specie* *Streptococcus faecalis* *Staphylococcus albus* *Staphylococcus aureus*	0.0	5	27.78
Bacillus subtilis *Corynebacteria specie* *Proteus mirabilis* *Staphylococcus albus* *Staphylococcus aureus*	0.5	5	27.78
Bacillus subtilis *Staphylococcus albus* *Corynebacteria specie* *Staphylococcus aureus*	1.0	4	22.22
Bacillus subtilis *Corynebacteria specie* *Staphylococcus albus* *Staphylococcus aureus*	1.5	4	22.22
Total		18	100

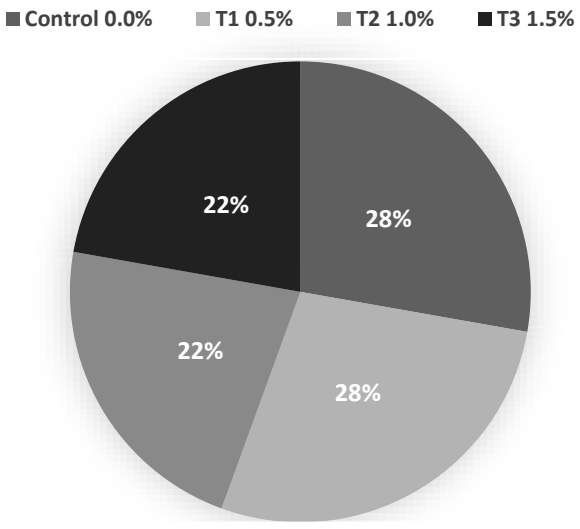

Figure 1. Percentage of bacteria isolated and identified in C. gariepinus smoked with Vernonia amygdalina extract at different concentration level for 21 days

in fish treated with 1.0% concentration the of extract followed by 10.39% in samples treated with 0.0% solution of the extract. The lowest value of moisture content was in sample treated with 0.5% of the extract (9.36%). The control samples recorded the highest crude protein content of 48.08% followed by samples treated with 1.5% solution with a value of 46.26%, while least value in fish treated with 0.5% of the extract at 39.67%. The least nitrogen free extract value of 23.97% was obtained in samples treated with 0.0% of the extract while samples treated with 1.0% had the highest nitrogen free extract of 24.95%.

Sensory Evaluation of Smoked *Clarias gariepinus* Treated with Different Concentrations of *Vernonia amygdalina* Extract

Table 5 shows the sensory evaluation of smoked-dried *C. gariepinus* treated with different concentrations of *V. amygdalina* extract. The mean scores of the sensory evaluation revealed that there was significant difference (P≤0.05) in the sensory evaluation among the treatments and the control, the control gave the best texture followed by the fish treated with 1% extract. The smoked-dried *C. gariepinus* retained good score for, appearance, colour, flavour and general acceptance after 21 days of storage and the overall acceptability mean score in all the treated samples and the control indicated that, the product was generally accepted.

Discussion

The result of the weight loss of *C. gariepinus* treated with different solution of *V. amygdalina* extract before and after smoking for 12 hours was presented in (Table 1). The percentage of average weight loss of 63.75% obtained in this study was similar to the percentage of average weight loss of 63.34% reported by Agbabiaka *et al.* (2012) in their research work on the nutritional and storage quality of catfish (*C. gariepinus*) smoked with *Anthonotha macrophylla*.

Bacterial population was high in the control 25×10^3 followed by 23×10^3 in sample smoked with 0.5% concentration of the extract. Least value was recorded in sample smoked with 1.5% concentration of the extract with value 9×10^3. In the changes in microbial loads of smoked fish products during storage at room temperature for the period of twenty-one (21) days, the result agreed with work of Martin *et al.* (2010). After one week of storage, the population of bacterial decrease in all the treated samples except in control which increase from 27×10^3 to 37×10^3 cfu. After (21) days of storage, decreased in microbial loads was recorded in all levels of concentration except for the sample treated with 0% extract concentration (control) which increase from 38×10^3 to 40×10^3 cfu, this could be due to the antimicrobial effect of the *V. amygdalina* extract on the smoked-dried fish. *Clarias gariepinus* treated with 1.5% solution of the bitter leaf extract was recorded the lowest in microbial loads 7×10^3 after twenty-one (21) days of storage. From the all samples of smoked fish examined, the result revealed presence of bacteria in all the samples. The different types of bacteria isolated and identified were *Bacillus subtilis, Corynebacteria species, Proteus mirabilis, Streptococcus faecalis, Staphylococcus albus* and *Staphylococcus aureus.* These micro-organisms may have contaminated the smoked-dried *C. gariepinus* through human handling or air. The presence of this micro-organisms in smoked fish samples might be due to increase in moisture content of the product during storage which favours the growth of this organisms as documented by Eyo (2001).

Table 4. Proximate composition of smoked-dried *Clarias gariepinus* treated with different concentrations of *Vernonia amygdalina* extract

	Vernonia amygdalina extract concentrations (%)			
Parameters	0.0	0.5	1.0	1.5
Moisture	10.39±0.02^c	9.36±0.02^c	11.35±0.04^b	10.09±0.02^c
Crude protein	48.08±0.82^a	39.67±0.04^c	43.70±0.04^b	46.26±0.01^d
Ether extract	6.00 ±1.63^b	7.00 ±1.63^b	9.00 ± 2.45 ^{a}	9.00 ±1.65^a
Ash	12.00 ±2.45^a	10.00 ±0.82^b	11.0±2.45^a	10.02.0±1.63^b
Nitrogen free extract	23.97±0.00^b	33.97±0.00^a	24.95±0.00^b	24.63±0.82^b

Mean values with the same superscript in a row are not significantly different (P>0.05)

Table 5. Sensory evaluation of smoked Clarias gariepinus treated with different concentrations of Vernonia amygdalina extract

	Concentrations *V. amygdalina* Extract (%)			
Parameters	0.0	0.5	1.0	1.5
Appearance	6.30±0.46^a	5.50±0.81^b	6.80±0.40^a	5.60±1.02^b
Colour	6.00±0.63^a	5.50±0.81 ^{a}	6.60±0.49^a	5.60±0.80^b
Flavour	6.00±0.63 ^{a}	6.00±0.63^a	6.20±0.75^a	5.60±0.80^b
Texture	6.20±0.87^a	5.90±0.94^b	6.20±1.16^a	5.70±1.01^b
General acceptance	6.60±0.49^a	6.30±1.00^a	6.70±0.46^a	6.20±0.60^a

The Value are the 7point Hedonic scale of 10 men panel response to each attributes. The Hedonic scale are 7=Excellent, 6=Very good, 5=Good, 4=Fair, 3=Poor, 2=Very poor and 1=Extremely poor. Mean values with the same superscript in a row are not significantly different (P>0.05).

The results of proximate composition indicated that, the crude protein formed the largest quantity of the dry matter among all treatments and the control, this was in line with the report of Ajani *et al.* (2013) who reported that, crude protein formed the largest quantity of dry matter in all fish. Also there was significant difference ($P<0.05$) among all the treatments and the control, the control sample recorded the highest crude protein content of 48.08% followed by samples treated with 1.5% solution with 46.26%. The lowest value of crude protein content was recorded in fish sample smoke in 0.5% of the extract at 39.67%. Morris (2001), stated that, protein and ash content do not vary as often as fat. There was no difference in ash content for all the samples but ash content of sample treated with 0.5% of the extract was much lower. Olayemi *et al*. (2011) stated that ash is a measure of the mineral content of fish.

The mean scores of the sensory evaluation revealed that there was significant difference ($P\leq0.05$) in the sensory evaluation among the treatments and the control, the control gave the best texture followed by the fish treated with 1% extract. The significant difference observed in this research could be due to the bitterness of *V. amygdalina* extarct used. However, the smoked-dried *C. gariepinus* retained good score for, appearance, colour, flavour and general acceptance after 21 days of storage and the overall acceptability mean score in all the treated samples and the control indicated that, the product was generally accepted.

Conclusions

Six bacterial species namely, *Bacillus subtilis, Corynebacteria sp, Proteus mirabilis, Streptococcus faecalis, Staphylococcus albus* and *Staphylococcus aureus* were observed in the study. There was no increase in the microbial loads in all the treatments except in the control which increased from 27×10^3 to 40×10^3 cfu. Highest crude protein content (48.08±0.82%) was recorded in the control while the least value (39.67±0.04%) was obtained in the fish treated with 0.5% concentration of the extract. Sensory evaluation revealed that, the smoked-dried *C. gariepinus* retained good score of overall acceptability. This study reveals that *V. amygdalina* extract can be used as preservative in protecting smoked-dried *C. gariepinus* from bacterial spoilage, thus limiting economic loss and possible health risk to consumers. Therefore, it is recommended to be used as natural preservative in fish processing and preservation industries.

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Author Contribution

Dr. A. I. Abdullahi and Associate Prof. M. M. Bello contributed equally to this research article.

Conflict of Interest

The authors declare that they have no conflict of interest in this paper.

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