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Antimicrobial Potentials of *Costus afar* Stem and *Ipomoea batatas* Leaf Extracts on the Preservation of *Oreochromis niloticus* Fish

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Abstract: *Fish is among foods that are susceptible to tissue decomposition, development of rancidity, and microbial spoilage. In this current study, the antimicrobial potentials of Costus afar stem and Ipomoea batatas leaf extracts on the preservation of Oreochromis niloticus stored for 48 hours were examined. The stem of Costus afar and the leaf of Ipomoea batatas were washed, crushed, squeezed and sieved off and a clear juice was obtained. The fresh O. niloticus was dipped into these prepared juice treatments (Treatment 1 = Costus afar; Treatment 2 = Ipomoea batatas; Treatment 3 = Costus afar + Ipomoea batatas; Treatment 4 = NaCl) samples for 20 minutes. They were observed every 4 hours for 48 hours. They were subjected to proximate analysis and microbial evaluation. From the result of the analysis chemical composition (Moisture content, dry matter, ash, ether extract, crude protein content, and carbohydrate) showed a significant difference (P < 0.05). Treatment 2 (Ipomoea batatas) had the best result in all the analyses, performing far better than the control. Also, observations were made in the microbial analysis in which treatment 2 had the lowest microbial load count and also being better than treatment 4 (Control – NaCl).*

Keyword: *Antimicrobial, Costus afar Stem, Ipomoea batatas Leaf, Oreochromis niloticus Fish*

INTRODUCTION

Fish preservation is the method of extending the shelf life of fish and other fishery products by applying the principles of chemistry, engineering, and other branches of science to improve the quality of the products (Wafaa *et al*., 2011; Adebayo-Tayo *et al*., 2012). Preservation methods material as an attempt to keep the fresh state so that the changes in texture and structure are minimized (Clucas, 1981; Amit *et al*., 2017). Fisheries make an important contribution to the animal protein supplies of many communities in both the industrialized and developing worlds (Adewolu and Adeoti, 2010). Although, theoretically, these proteins and nutrients could come from other sources, in isolated fisheries-dependent communities, alternatives are likely to be more expensive, if they are available at all (FAO, 2010). Fish is a highly perishable commodity, more

than cattle, sheep, poultry get spoiled very quickly after capture. Food preservatives and anti-oxidants are used to prolong the shelf life of food either by killing microorganisms or controlling their growth in food (Surekha and Reddy, 2014). They also preserve by preventing or retarding the oxidative deterioration of food (Ligia, 2002). Synthetic anti-oxidants have been prohibited in many countries of the world because of their undesirable effect on the enzymes of the liver and lungs (Ikeme and Bhandary, 2001). This has paved the way for the extensive use of natural preservatives and anti-oxidants such as spices, fruits (stems, peels and seed) and other plants for the preservation of spoilage, mainly by microorganism (that naturally inhabit the fish flesh) in fresh and smoked fish (Amit *et al*., 2017; Teshome *et al*., 2022). Many plants are today being scientifically studied for bioactive substances and medicinal potentials, *Costus afar* appears to be among the least evaluated and as such has scanty published literature on its physiological and pharmacological dynamics (Anyasor *et al*., 2014). The plant is commonly called Ginger lily or Bush cane in English, Kaki zuwa by the Hausas, Okpete or Okpoto by the Igbo and TeteOgun by the Yoruba, all in Nigeria (Anaga *et al.*, 2004; Ukpabi *et al*., 2012), it is a tall perennial semi-woody herb with leafy canes which may grow up to 3m high and belonging to family Costaceae (Bukil, 1985). *Costus afar* bears a terminal inflorescence of white and yellow flowers and is commonly found in the forest zones of most places including Senegal, Nigeria, South Africa, Guinea, Ghana, and Cameroun and most regions in tropical Africa, particularly in higher rainfall areas (Bukil, 1985; Ukpabi *et al*., 2012). *Ipomoea* is the largest genus of the flowering plant family convolvulaceeae with over 500 species. The leaves of *I. batatas* are eaten as a vegetable and have been shown to slow oxygenation, with some similar potential health benefits as green tea. Fresh fish is one of the most perishable food staples. In the tropics, fish spoil within 12-24 hours depending on the species and method of capture (Clucas and Ward, 1996). The high ambient temperature in the tropics hastens fish spoilage by accelerating the activities of bacteria, enzymes and chemicals oxidation of fat in fish flesh (Eyo, 2001). The amount of post-harvest losses has led to a decline in fish supply and the human population keeps rising. It is therefore very necessary to assess the use of some medicinal plants such as *Costus afar* and *Ipomoea batatas* as a preservative to extend the deteriorating/perishing life of fresh fish before it is sold. Given that about 40% of fish is lost to post-harvest spoilage (FAO, 2000), an adequate preservation method must be found such as the use of *Costus afar* and *Ipomoea batatas* for the preservation of early catches of fish by the fisher folk until the desirable market becomes available. This study was therefore undertaken to determine the preservative effects of *C. afar stem* and *I. batatas* leaf extracts on fresh *O. niloticus.*

MATERIALS AND METHODS

Location of the study

The experiment was carried out at the Department of Fisheries and Aquatic Resources Management, Michael Okpara University of Agriculture, Umudike.

Preparation of leaf extract

The stem of *C. afar and* leaves of *I. batatas* were collected from the Michael Okpara University of Agriculture Teaching and Research Farm, washed, and ground fresh using a pestle and mortar. For the first and second treatment 500g of each were ground and extracted using 1 liter of water, for the third treatment 250g of each were ground and extracted using 500cl of water. The two extracts were mixed to form 1 liter of the mixture. Four extract treatments were allotted for this

current study, Treatment 1 (*Costus afar*); Treatment 2 (*Ipomoea batatas*); Treatment 3 (*Costus afar* and *Ipomoea batatas*), and Treatment 4 (Control) respectively.

Experimental fish and preservation using extract

The *O. niloticus* fish used in this study was collected from the Michael Okpara University of Agriculture Fish Farm. The fish was dipped inside the individual treatments in separate bowls of the stem and leaf extract classified under treatment for 20 minutes.

Proximate Analysis

The proximate composition of the fresh fish was determined by the association of an official analytical chemist (AOAC, 2000). This analysis was done before and after the experiment to ascertain the rate of deterioration of the crude protein (CP), crude lipid (CL), crude fiber (CF), carbohydrate, ash, and moisture.

Microbial Test

1gm each of the samples was ground in a mortar with pestle sterilized with 70% ethanol. This was added to 10ml of sterilized by autoclaving distilled water and shaken together. Serial dilution was made by adding 1 ml of the solution of 1gm of samples in 10 ml of distilled water. It was added to 9ml of sterilized water. 1 ml of the last dilution was added to 9 ml of sterilized distilled water. One more dilution was made further. These different dilutions were vigorously and allowed to be studied for 30 minutes. The number of bacteria in 1 ml of each dilution was determined by spreading a drop of the dilution on the nutrient agar surface. The number of drops in 1 ml of the solution from the pipette was determined. The inoculated nutrient agar plates were incubated for 18-24 hours at 37^{o}C. The number of colonies obtained was multiplied by the number of drops obtained in 1 ml of each dilution. The result multiplied by the dilution factor gives the bacteria count in 1gm of the fish sample.

Statistical Analysis

All data collected from the proximate analysis were subjected to analysis of variance (ANOVA) and means separation was done by the Duncan Multiple Range Test (DMRT).

RESULTS AND DISCUSSION

The proximate composition of fresh *O. niloticus* preserved with the stem extract of *Costus afar* and the leaf extract of *Ipomoea batatas* is presented in Table 1. The highest moisture content was recorded in *C. afar* (79.78±38). The least moisture was recorded in *I. batatas* 71.1+06. There was a significant difference ($p<0.05$) in all the treatments. The dry matter content was highest in *I. batatas* (28.9+0.06) and *C. afar* had the least dry matter content (20.22±0.38), This implies that the lower the moisture content, the higher the dry matter content. There was a significant difference ($p<0.05$) in the ash content between *C. afar* and *I. batatas* but there was no significant difference between treatment 2 and 4. The treatment with the highest ash content was the control (5.42±0.50) which had no significant difference with *I. batatas*, the least ash content was recorded in the *C. afar* (3.14±0.020). There was no significant difference in the fiber content in all the treatments.

Likewise, the Ether extract was highest in *I. batatas* (2.34±0.02) and lowest in *C. afar* (2.14±0.00). There was no significant difference in the Ether extract in treatments 2, 3, and 4. The result also revealed in the table shows that *I. batatas* have the highest crude protein content (15.12±0.05) and the least was recorded in treatment 1. Furthermore, there was a significant difference ($p<0.05$) in all the treatments. For the carbohydrate, *I. batatas* had the highest (5.11±0.16), while *C. afar* had the lowest (2.02±0.10).

The result from Table 2 shows that there was a significant difference in the coliform count (log10 cfu/g) for the fresh *O. niloticus* preserved with *Costus afar* stem and *Ipomoea batatas* leaf extract. The microbial load was highest in treatment I (*Costus afar*) recording 2.35 xlO7, followed by treatment 3 (*Costus afar + Ipomoea batatas*) with a count of 2.18 x 107, then treatment 4 (control) 1.96 x 107, the least is treatment 2 *(Ipomoea batatas*) with a microbial load count of 1.64 xlO7. This difference could be a result of the leaf attracting more microorganisms. The microbial identified in this current study were; Proteus, *Pseudomonas sp*., *Enterobacter*, *Klebsiella sp.*, *Bacillus sp.*

Table 1: shows the mean value of the proximate composition of the test fish (*O. niloticus*) samples

Parameters	Treatment 1	Treatment 2	Treatment 3	Treatment 4
Moisture content	79.78 ±0.38^{a}	71.1 ± 0.06^{d}	78.02 ± 0.22^{b}	72.36 ± 0.08^{c}
Drymatter	20.22±0.38^{d}	28.9±0.06^{a}	21.99±0.22^{cc}	24.44 ± 0.07^{b}
Ash	3.14 ±0.02^{d}	5.27 ± 0.02^{b}	3.32±0.04^{c}	5.42 ± 0.50^{a}
CrudeFibre	1.03 ±0.01^{a}	1.08 ±0.02^{a}	1.04±0.01^{a}	1.06±0.02^{a}
Ether Extract	2.14±0.00^{d}	2.34 ± 0.02^{a}	2.23 ± 0.03^{bc}	2.28 ± 0.20b
CrudeProtein	12.19±0.01^{d}	15.12±0.05^{a}	12.69±0.09^{c}	13.58±0.40^{b}
Carbohydrate	2.02±0.10^{d}	5.11±0.16^{a}	2.71 ± 0.39^{c}	4.32±018^{b}

Treatment 1 = *Costus afar,* Treatment 2 = *Ipomoea batatas,* Treatment 3 = *Costus afar + Ipomoea batatas,* Treatment 4 = Control

Table 2: Microbial analysis of the test fish samples during the 48 hours of storage period Mould Count cfu/g

Hours	Sample			
	T1	T2	T3	T4
0 hr	0	0	0	0
48 hrs	2.35 xlO^{7}	1.64xl0^{7}	2.18 xlO^{7}	1.96xl0^{7}

Treatment 1 = *Costus afar,* Treatment 2 = *Ipomoea batatas,* Treatment 3 = *Costus afar + Ipomoea batatas,* Treatment 4 = Control

Conclusions

The results of this study suggest that *Costus afar* stem and *Ipomoea batatas* leaf extracts can be utilized as natural preservatives in the fish industry. The extracts offer promising alternatives to synthetic preservatives, as they not only possess antimicrobial and antioxidant activities but treatment 2 (*Ipomoea batatas* leaf extracts) had the best antimicrobial and antioxidant activities

on the test sample (*Oreochromis niloticus*) far better than treatment 4 (Control – NaCl). *Costus afar* stem and *Ipomoea batatas* leaf extracts can be considered as viable natural alternatives to synthetic preservatives in the fish industry. The use of these extracts could help extend the shelf life of *Oreochromis niloticus* fish while maintaining its quality. While this study provides promising results, further research is necessary to investigate the exact mechanisms of action of the extracts and their potential application in other seafood products.

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