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Effects of *Vernonia amygdalina* and *Allium sativum* (Linn.) on microbial content of smoked *Clarias gariepinus* (Burchell, 1822)

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Abstract

This study was conducted to evaluate the effects of bitter leaf *Vernonia amygdalina* and garlic *Allium sativum* on microbial content of freshly smoked *Clarias gariepinus*. Sixteen table-size of life *C. gariepinus* with average weight and standard length of 287.0g and 34.3cm, respectively were purchased from Sabon-gari market, Zaria, Kaduna State. They were sorted into 4 groups, smoked and treated with garlic and or bitter leaf as follows: Smoked with garlic (SWG), Smoked with bitter leaf (SWB), Smoked with bitter leaf and garlic (SWB&G), while a group smoked without treatment (SWT) served as the control. Duplicate samples per group were tested for microbial loads, at the end of 24 and 72 hrs following recommended methods of the International Commission of Microbiological Specifications for Food. Some of the bacteria identified were *Streptococcus* species, *Staphylococcus aureus* and *Staphylococcus epidermidis*, while the fungi were *Aspergillus niger*, *Fonsecaea* spp., *Trychophyton* spp. and *Malbranchea* species. The bacterial load for the various treatments (SWT, SWG, SWB and SWB&G) showed significant differences ($P \leq 0.05$). The mean number of colony forming units for SWT was 292, SWB was 44, SWG was 132, and SWB&G was 70. SWB showed growth of a colony of *Aspergillus niger* while SWB&G showed growth of 5 colonies of *Fonsecaea* spp., *Trichophyton* spp. and *Malbranchea* spp. This study shows that bitter leaf has significantly higher ($P \leq 0.05$) antimicrobial effect than garlic and is thus recommended for reducing microbial load on smoked *Clarias gariepinus*.

Keywords: Bitter leaf, *Clarias gariepinus*, garlic, microbial load and smoked.

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Introduction

Fish is a major source of food for human, it provides significant portion of the protein intake in the diets of a large proportion of people, particularly in the developing countries. Fish is one of the cheapest sources of protein that is used to correct protein deficiency in the tropics (Akinwumi, 2011). Fish is a major source of animal protein in Nigeria. It is readily available in most markets as fresh, smoked, dried, canned, chilled or frozen. Smoking of fish is a traditional method of processing fish around the globe, thereby extending the shelf-life of the smoked fish. Although its acceptance is based on the sensory characteristics it imparts on the fish products.

Fish smoking in the tropics is conducted in smoke houses and smoking ovens or kilns with varying equipment and designs (Eyo, 2001). Smoking kilns can be classified into three: the traditional, the improved traditional and the mechanical smoking kilns. Heat generated by smoking process removes water, inhibits bacterial growth, retards enzymatic action, and imparts aroma, taste and color on processed fish. However, fish quality can deteriorate during

storage due to lipid oxidation and microbial growth; lipid oxidation is responsible for reduction in nutrients quality as well as changes in flavors, while microbial contamination could cause public health concern as well as economic and physical loss in terms of fish spoilage (Kumolu-Johnson and Ndimele, 2001). The bacteria that are most often involved in the spoilage of fish are part of the natural flora of the external slime of fishes and their intestinal content (Ibrahim *et al.*, 2011).

Allium sativum (garlic) is one of the commonly used natural ingredients to enhance flavor in food. It has been reported to have a wide spectrum of actions which include antibacterial, anti-fungal and anti-oxidative. It has beneficial effect on cardiovascular and immune system of human (Sallam *et al.*, 2004). *Vernonia amygdalina* (bitter leaf) is a shrub that grows predominantly in the tropical Africa. Leaves from the plant serve as vegetable food and culinary herb in soup (Abosi and Raseroka, 2003). Flavonoids obtained from *V. amygdalina* have high antioxidant activities and its saponins have been reported to elicit antitumoral activities in leukemia cells (Iisaka *et al.*, 1993).

Materials and Methods

Collection and Preparation of Samples

Sixteen table-size samples of live *Clarias gariepinus*, fresh bitter leaves and garlic cloves were bought from Sabon-gari market, Zaria and transported to the Department of Biological Sciences, Ahmadu Bello University, Zaria. The bitter leaves and garlic cloves were then sun-dried and ground. The hot smoke method was employed with an exotic hard wood (*Eucalyptus* species). Smoking of fish was done for 1 hour, turning of the fish species were done at the same time to maintain uniform smoke/drying at an intervals of 10-15 minutes. Flesh (10g) of the smoked fish samples were sorted into 4 groups and treated accordingly: Smoked without treatment (SWT), Smoked with garlic (SWG), Smoked with bitter leaf (SWB), Smoked with bitter leaf and garlic (SWB&G). Samples of flesh from each group were cut and transferred to 90 ml of 0.1% buffered peptone water (Difco) and the treatments (Powders of bitter leaves and garlic cloves) were added in sterile flask and homogenised to make a 1:10 M/V dilution. The homogenate was thoroughly mixed before making serial dilution(s) according to International Commission of Microbiological Specifications for Food (ICMSF, 2012). After vigorous shaking, serial dilutions were carried out from which 1ml each of dilution factors 10^{-2} and 10^{-3} were plated using a sterile syringe into the petri dishes containing the appropriate agar. The dilutions were plated in duplicates and incubated at 37$\pm$2ºC for 24 hours for bacteria and at 37$\pm$2ºC ºC for 96 hours for fungi.

Media preparation

All analytical procedures in this study are according to recommended methods of Association of Analytical Chemists (2002). Nutrient Agar, MacConkey Agar, Potato Dextrose Agar (PDA), Mannitol Salt Agar (MSA) and Salmonella Shigella Agar (SSA) were prepared following the manufacturers' instructions which involved dissolution of weighted quantities of the Agar by bringing to boil, distribution into Mac Cartney bottles and autoclaving at 121ºC for 15 mins.

Gram Staining and Biochemical Tests

The slides were cleaned with cotton wool immersed in alcohol in order to get rid of greasy stain, the wire loop was sterilized over Bunsen burner flame until it was hot red, it was then allowed to cool, after cooling, a colony was picked from MSA culture and placed on the slide containing a drop of distilled water and a smear was made. It was left to air dry before fixing the slide using heat. After heating, the slide was flooded with crystal violet and left for 60secs, rinsed with water, flooded again with grams iodine and left for another 60secs, and then decolourised with acetone for 30secs to avoid over decolourisation it was counter stained with safranin and left for 60secs, before rinsing with water. The slide was cleaned and allowed to air dry and then viewed under the microscope to view the microorganisms (Cowan and Liston, 1974). Catalase test using 3% hydrogen peroxide via the slant method, and enzyme coagulase test were conducted for further identification of

microorganisms isolated (Harrigan and Mac Cane, 1976).

Results

The total bacterial count in smoked *Clarias gariepinus* without and with treatments are shown in Table 1. The mean number of colony of SWT was 2.92 x 10^{-2}, SWB was 4.4 x 10^{-1}, SWG was 1.32 x 10^{-2}, SWB&G was 7.0 x 10^{-1}. The SWT, SWG, SWB and SWB&G show significant differences ($P \leq 0.05$) in total bacterial count.

The SWT had the highest total bacterial count. Bacteria identified in fish sample of smoked *Clarias gariepinus* include *Staphylococcus aureus*, a gram-positive bacterium which had yellow growth colonies on Mannitol Salt Agar at 18-24 hours and was reactive to Coagulase test and Catalase test. *Staphylococcus epidermidis*, a gram-positive bacteria, produced red colonies on MSA at 24-48hrs, Coagulase test was non-reactive, Catalase test was reactive. *Streptococcus* spp. were gram-positive, had no growth on MSA but on EMB and Mac Conkey Agar, and was non-reactive to Catalase test.

Total fungi (TFC) for smoked *Clarias gariepinus* without and with treatments are shown in Table 2. SWT had no fungal growth, SWG also had no growth, SWB had growth of a colony (*Aspergillus niger*, Plate I), SWB&G had growth of 5 colonies (*Fonsecaea* spp., *Trichophyton* spp. and *Malbranchea* spp.

Table 1: Total bacterial count for smoked *Clarias gariepinus* treated with bitter leaf and garlic

Sample Code	Number of Colony	Population in Colony Forming Unit/g
SWT	292 ± 160.00 [a]	2.92×10^2
SWG	132 ± 12.00 [b]	1.32×10^2
SWB	44 ± 8.00 [d]	0.44×10^2
SWB&G	70 ± 10.00 [cd]	0.77×10^2

Note: Means with different superscripts along the column are significantly different ($P \leq 0.05$) dilution factor 10^{-3}
Key:
SWT: Smoked without treatment
SWG: Smoked with garlic
SWB: Smoked with bitter leaf
SWB&G: Smoked with bitter leaf and garlic

Table 2: Total fungi count (TFC) for smoked *Clarias gariepinus*

Sample Code	Number of Colony	Population in Colony Forming Unit/g
SWT	292± 160.00 [a]	2.92×10^2
SWG	132 ± 12.00 [b]	1.32×10^2
SWB	44 ± 8.00 [d]	0.44×10^2
SWB & G	70 ± 10.00 [cd]	0.77×10^2

Sample Code	Number of Colony	Population in cfu/g	Fungi suspected
SWT	nil	nil	nil
SWG	nil	nil	nil
SWB	1	0.1×10^1	*Aspergillus niger*
SWB & G	5	0.5×10^1	*Fonsecaea* spp., *Trichophyton* spp. and *Malbranchea* spp.

Note: Dilution factor 10^{-2}
Key:
SWT: Smoked without treatment
SWG: Smoked with garlic
SWB: Smoked with bitter leaf
SWB&G: Smoked with bitter leaf and garlic

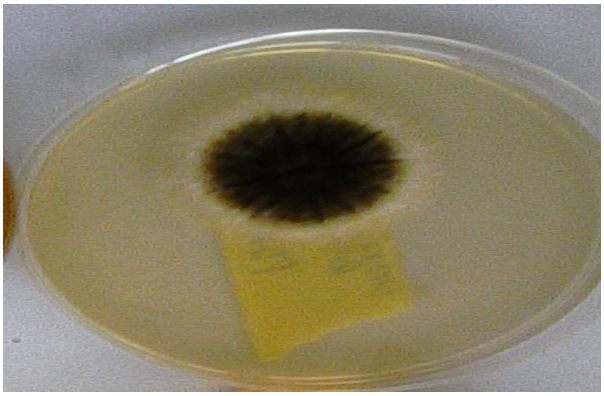

Plate I: *Aspergillus niger* growing on PDA

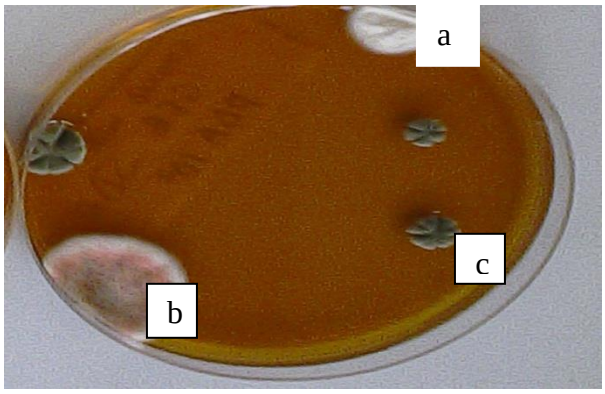

Plate II: *Malbranchea*(a) spp., *Fonsecaea*(b) spp. and *Trichophyton* (c) spp. growing on PDA

Discussion

The smoked *Clarias gariepinus* without treatment has the highest number of bacterial and fungal colonies, though smoking has been reported to pose bacteriocidal activity. The high microbial load probably resulted from unhygienic handling or pre-infected smoking surfaces during processing, since most of the bacteria are normal skin flora not originally found on the fish. These bacteria are *Staphylococcus aureus* and *Staphylococcus epidermidis*. Abolagba and Iyeru (1998) reported that improper smoking and unhygienic handling of smoked fish results in high microbial infestation.

The smoked fish with garlic has low microbial count compared with the smoked fish without treatment; this may be due to the presence of allicin an organosulfur compound obtained from converted alliin by the enezyme alliinase, which confer bactericidal properties to garlic (Curtis *et al*., 2004). Garlic extract has been reported to exhibit broad spectrum antimicrobial activity at room temperature (Sivam *et al*., 1997). Garlic has been reported to contain antioxidative and antimicrobial substances (Sallam *et al.*, 2004). The smoked fish with bitter leaf and garlic has synergistic effects by having greater bactericidal effect compared to smoked without treatment at $P \leq 0.05$. Bitter leaf imparted significant ($P \leq 0.05$) bacteriostatic action than the other treatments on the smoked fish.

Smoked without treatment had no fungal growth, this may be due to the method of preservation. The smoked with bitter leaf do not pose significant anti-mycotic action and also the environmental humidity may have favoured the fungal growth. Abolagba and Iyeru (1998) reported that high humidity supports the growth of moulds in stored food. Moulds produce mycotoxins, some of which are carcinogenic (Zain, 2011). Fish smoked with both bitter leaf and garlic has no effect on the fungal growth. The prevalence of fungi compared to bacteria may be attributable to the small particle size of the ground garlic and bitter leaf necessary for homogenization. Bacteria growth has been reported to be especially affected by fragment size and fungi penetrate such small particles of substances more easily than bacteria (Fogel, 2003). It may also be attributed to the lower minimum water activity (Aw) requirement for fungi growth (0.7) compared to bacteria (0.91).

References

Abosi, A.O. and Raseroka, B.H. (2003). In-vivo antimalarial activity of *Vernonia amygdalina. BJ Biomedical Science*, 60:89-91.

Agbolagba, O.J. and Iyeru, O.A. (1998). Study of insect pest infecting traditionally processed fish sold in Benin City metropolis. *Nigerian Journal of Applied Science, 16: 25-29.*

Akinwumi, F.O. (2011). Evaluation of some plant materials for the control of smoked fish pest, *Dermetes maculatus* Degeer (Coleoptra: Dermestidae) in *Clarias gariepinus* Burchell (Pisces:Clariidae). *APRN Journal of Agricultural and Biological Science*, 6(7): 65-69.

Cowan, S.T. and Liston, J. (1974). The mechanism of identification, p. 10-13. In: R.E Buchanan and N.E Gibbons (ed), *Bergey's Manual of Determinative Bacteriology*, 8th edition. The Williams and Wilkins Co., Baltimore.

Curtis, H., Noll, U., Stormann, J. and Slusarenko, A.J. (2004). Broad-spectrum activity of volatile phytoanticipin allician in extracts of garlic (*Allium sativum* L.) against plant pathogenic bacteria, fungi and oomycetes.

Eyo, A. A. (2001). *Fish Processing Technology in the Tropics*. National Institute for Freshwater Fisheries Research (NIFFR),"

New Bussa. Published by the University Of Illorin Press., pp. 10-170.

Fogel, R. (2003). Fun facts about fungi, Utah state university Intermountain Herbarium. www.herbarium.usu.edu/fungi/factindx

Harrigan, W.F. and McCane, M.E. (1976). *Laboratory Methods in Food and Dairy Microbiology*, Academic Press, London, UK. Pp 19-20.

ICMSF (2012). Microorganisms in foods: microbiological testing in food safety management. International Commission on Microbiological Specifications for Foods.

Ibrahim, A. A., Kigbu and Muhammed, R. (2011). "Women's experiences in small scale fish processing in Lake Fafenwa, fishing community, Nassarawa State," *Nigeria Livestock Research for Rural Development*, 23:3-5.

Iisaka, M., H., Ohigashi, K., Takegawa, M., Hirota, R., Irie, M.A., Huffman and Koshmizu, K. (1993). Steroid gluccosides from *Vernonia amygdalina*, a possible chimpanzee medicinal plant. *Phytochemical*, 34: 409-413.

Kumolu-Johnson, C.A and Ndimele, P.E. (2001). Effect of salting, brine and sun-drying on the shelf life of *Clarias gariepinus. Journal of Research Review in Science*, 2:21-25.

Sallam, K.H.I, Ishioroshi M.I and Samejima. (2004). Antioxidant and Antimcrobial effect of garlic on chicken sausage, *Lebson Wiss Technol*, 37(8):849-855.

Sivam, G.P., Lampe, J.W., Ulness, B. Swanzy, S.R and Potter, J.D. (1997). *Helicobacter pylori* in-vitro susceptibility to garlic (*Allium sativum*) extract. *NutrCancer*, 27(2):118-121.

Zain, M.E. (2011). Impact of mycotoxins on humans and animals. *Journal of Saudi Chemical Society*, 15(2):129-144.