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EVALUATION OF ANTIMICROBIAL PROPERTIES OF FOUR SPICES AGAINST STRAINS OF BACTERIA AND OTHER ORGANISMS CAUSING SPOILAGE TO STORED FISH

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ABSTRACT
Evaluation of antimicrobial properties of the four spices against strains of bacteria and other organisms causing spoilage to stored fish Was carried out at the Joseph Sarwuan Tarka University Makurdi formerly University of Agriculture Makurdi in the Department of fisheries and aquaculture, September 2020 to January 2021. Using Xylopia aethiopica, (African pepper, Negro pepper, igyangyase), Monodora myristica (African nutmeg, Ehuru), Piper guineense (West African pepper, uziza, iyere, yiye), Tetrapleura tetraptera (Aidan tree, gum tree, uhio, kyoho) were used at a concentration of 90g which was selected to be the most effective after the pre test at different dossage. A total of (32) freshly caught fish, with similar weight of 0.5kg were purchased from lower Benue (Wadata market) the two species (Clarias gariepinus and Synodontis schall) weighing about 0.5kg each. The 90g spice extract were dissolved in 500ml of water and left overnight for proper infusion of the compound. And the fish was immersed in the spice and smoke dried using charcoal powered oven after which the smoked fish were analysed for microbial load before storage using the traditional method in the microscopic examination of bacteria in the Biotechnology laboratory of Sheda Science and Technology complex using the grams staining method. The data obtained from the experiment was subjected to Analysis of Variance and Mean Separation. The result of the microbial activities of catfish before and after storage showed that apart from control and Gum tree all other varriables were significantly different. There was a drop in the microbial load after storage as can been in African nutmeg before storage (57.50±6.10) and after storage (34.20±4.70) same applied to African pepper gum tree and west African pepper both at CFU/ml (100) and (10-2). Synodontis schall showed significant difference only in African pepper treated spice but also showed a decrease in microbial load African nutmeg before (87.30±6.80) and after storage (68.7±4.30) this was same in the other spices both at (10^⁰)and (10-2).The reduction of the microbial activities and microbes on the fish signify the inhibitory properties of spices and this means fish can be kept safe for longer times using spices as preservatives.

Keywords: Antimicrobial, Spices, Bacteria and Strains.

1. INTRODUCTION
Traditionally, fish is considered an affordable source of protein of high biological value, including high levels of essential amino acids (lysine, methionine, etc.), lipid-soluble vitamins, minerals (Se, P, Fe, Mg, and K), and rich in highly unsaturated fatty acids (ω3, 6, and 9), such as docosahexaenoic and eicosapentaenoic acid (Das *et al.,* 2021). The lipid fraction and other bioactive components present in fish, have attracted a great deal of attention because of their

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favorable effects on human health including reducing the risk related to human cardiovascular and chronic neurodegenerative diseases (Khalili, 2018). Accordingly, the World Health Organization and American Heart Association have also recommended consuming 1−2 servings of fish weekly regularly (Kontominas *et al*., 2021).

Fish are usually dehydrated as a means of preservation, as fish is well-known for its perishability issue. For regions that have easy access to large areas of water, fish is a major source of nutrients for their community. In addition, dried fish differs from place to place and culture to culture, from the type of fish used to the drying methods and ingredients used in the drying process (Bao *et al*., 2020)

The nutritional value of fish and shellfish protein is not inferior to eggs, meat, and other high-quality proteins. The lipids of aquatic animals have fluidity at low temperatures and are rich in polyunsaturated fatty acids and non-triglycerides, which are quite different from those of terrestrial animals. However, fresh aquatic products are highly susceptible to spoilage in the presence of endogenous enzymes and microorganisms. In addition, unpleasant odors and harmful substances are produced during deterioration. The action of microorganisms is the main reason for the deterioration of aquatic freshness and quality during storage (Stemisa et al., 2020). With the prolongation of storage time, the growth and metabolism of microorganisms also become gradually active, especially some specific spoilage organisms (SSO), which produce various proteases and lipases to decompose nutrients, such as proteins and lipids. In addition, the fishy odour is an important problem in the processing of aquatic products. The formation of fishy odor compounds is influenced by many factors, including the degradation of endogenous enzymes, lipid oxidation, growth of microorganisms and environmental pollution (Liu et al., 2021)

Incorporating synthetic antioxidants viz., BHT, BHA, TBHQ at high doses, either to the raw materials or end-products can negatively affect human health (Venugopal and Gopakumar, 2017). Prolonged use of these synthetic preservatives and compounds are reported to induce cancer, liver and kidney damage, gastrointestinal disorders, asthma, and many allergies (Etemadi *et al*., 2017). With increased concerns and negative perceptions among consumers regarding the safety aspects of chemical preservatives and synthetic compounds, the demand for minimally processed ready-to-eat fish products has increased manyfold. To cater to the demand of consumers and to ensure the availability of safe, nutritious, tasty, and convenient food, natural alternatives, such as those involving phytochemicals/phytoextracts, including essential oils, phenolics etc. derived from plants have gained lots of interest. Plants and their parts harbor a complex mixture of bioactive compounds that are a good source of phytochemicals such as polyphenols, phytosterols, alkaloids, nitrogen-containing compounds, terpenoids, organosulfur compounds etc. These compounds possess inherent biological effects such as antimicrobial, antioxidant, antidiabetic, anti-inflammatory, immune-enhancing functions etc. that are reported to offer health benefits (McClements *et al*., 2021)

Spices have been widely used since ancient times and have become an important part of the food habits of most population groups worldwide (Bao *et al*., 2020) Over 150 different types of spices are reported worldwide. A total of 109 are certified by the International Organization for Standardization (ISO) 676: 1995. The primary role of spices is not only to improve food flavor but also to use different colors, such as black, green, red, and brown. The color of spices can make people enjoy real pleasure in the senses and directly affect the digestion and absorption of food (Sun et al., 2024). In addition, spices are also known for their preservative, antioxidative, and antimicrobial properties (Yan *et al*., 2024). Spices can not only improve the flavor of aquatic products but also inhibit spoilage of aquatic products. They can effectively inhibit the oxidation of muscle protein and lipids, inhibit the growth and reproduction of microorganisms, and delay corruption and deterioration, thus maintaining the quality of aquatic products and extending their shelf life (Ismal *et al*., 2016)

As the focus of the processing industry is to maintain the organoleptic and nutritional characteristics and ensure the quality and safety of food products, the use of plant related preservative is gaining increasing importance nowadays, many individuals turn to show interest in the consumption of organic food than chemical food. This work is aimed at evaluating the antimicrobial properties of four spices against strains of *bacteria* and other organisms causing spoilage to stored *Clarias gariepinus* and *Synodontis schall* which are commercially important fish species in west Africa.

2. MATERIALS AND METHOD

Experimental Site

This experiment was carried out at the Department of Fisheries and Aquaculture, Joseph Sarwuan Tarka University of Agriculture Makurdi, Benue State.

Experimental Design

All the four spices *Xylopia aethiopica*, (African pepper, Negro pepper, igyangyase), *Monodora myristica* (African nutmeg, Ehuru), *Piper guineense* (West African pepper, uziza, iyere, yiye), *Tetrapleura tetraptera* (Aidan tree, gum tree, uhio, kyoho) were used at a concentration of 90g.Before the main experiment, a preliminary test was carried out to determine the efficacy of each of the above mentioned spice at 30g, 60g and 90g. 90g had the best performance hence it was adopted for the work. Two fish samples (weighing 0.5kg) with three replicate were applied with a concentration of 90g and smoked to dry.

Collection of Samples

A total of (32) freshly caught fish, with similar weight of 0.5kg were purchased from lower Benue (Wadata market) the two species (*Clarias gariepinus and Synodontis schall*) weighing about 0.5kg each. Some dried seed quantity of African pepper, African nutmeg, West African pepper and Gum tree were purchased at Wurukum market Makurdi Benue State.

Preparation of Samples

The purchased seeds were washed, dried and ground into powder using a blending machine (Buchymix) and weighed to obtain a weight of 90g using a digital weighing scale. The fish samples weighing 0.5kg each were washed degutted and grouped into 4 treatments of 3 fish each (One from each specie). The 90g spice extract were dissolved in 500ml of water and left overnight for proper infusion of the compound *Monodora myristica* (African nutmeg), *Piper guineense* (West African pepper), *Xylopia aethiopica* (African pepper) and *Tetrapleura tetraptera* (Gum Tree). Each batch of the fish were immersed in the solvent of African pepper, African nutmeg, West African pepper and Gum tree respectively for 30 minutes coded A, B, C and D. Thereafter, fish samples were removed and put into separate baskets and covered with muslin cloth to drain for 15 minutes. After this time, the fish samples were arranged into oven trays and allowed to dry at temperature of $100^{o}C$-$105^{o}C$.

Processing Techniques

Drying was conducted by using charcoal powered oven. The pre-treated fish samples were arranged on the oven trays and allowed to smoke dry for 10 hours during which turning over of the fish was done at intervals to achieve uniformly smoke-dried product. Thereafter, the smoke-dried product were removed from the oven and arranged on trays and allowed to cool at room temperature before weighing in order to determine the moisture loss. Samples were labelled accordingly before transporting it to the laboratory to check the effect of the spice on the shelf life and microbial load of the fish by conducting microbial analysis. And carefully storing the rest for 16 weeks (Ayodeji *et al*., 2020)

Microscopic Examination of Microbes

The smoked fish were analysed for microbial load before storage using the traditional method in the microscopic examination of bacteria in the Biotechnology laboratory of Sheda Science and Technology complex using the grams staining method. The description of the staining method was extracted from (Cheesebrough, 2000), while the method of the microscopic study of fungi was conducted according to (Harrigan and Mclance, 1990).

Biological Evaluation

The following biological evaluation were performed on the samples before and after smoking Tougan *et al*., (2013).

i. Dressed Weight = Carcass Weight-Weight of Offal.

ii. Total Weight loss = live Weight/ Carcass Weight-Weight after smoking

% *weight loss = Total weight loss/ Live weight of fish* ×100

Statistical analysis

Statistical analysis was carried out using One-way Analysis of variance. The data obtained from the experiment was subjected to Analysis of Variance and Mean Separation (Duncan, 1955) using SPSS.

3. RESULTS

In table 1. The result showed the total microbial load before and after storage.

There was no significant difference in the control before and after storage. In African nutmeg treated fish there was a significant difference before and after storage with the lowest microbial load after storage (34.20×10^{o}). African pepper treated fish also differed significantly before and after storage with the lowest coliform count (CFU/ml(10)) after storage (63.00×10^{o}) Gum tree treated *Clarias gariepinus* did not differ significantly before and after storage but a low microbial load was observed after storage (85.00×10), West African pepper treated fish showed a significant difference before and after storage with lowest microbial load seen after storage .

Similar trend was observed in the same fish species control showed no significant difference before and after storage. African nutmeg treated fish showed a significant difference before and after storage with the lowest microbial load seen after storage (4.5×10^{-2}).Gum tree treated fish showed no signifant difference before and after storage but with a low microbial load seen after storage (30.30×10^{-2}). African pepper treated fish however, showed a significant difference before and after storage with the lowest mean seen after storage (12.83×10^{-2}), West African pepper treated fish followed the same trend showing a significant difference before and after storage with the lowest microbial load after storage (6.67×10^{-2}).

Table 1: Microbial evaluation of *Clarias gariepinus* before and after storage

Microbial Parameter/ Biopreservatives	Time of Storage Before Storage	After Storage	Df	T-Value	P-Value	Percentage (%) effect
CFU/ml (10^0)						
Control	128.50±1.20	124.00±0.71	5	0.71	0.74	3.50
African Nutmeg	57.50±6.10	34.20±4.70	9	3.02	0.01*	40.00
African pepper	114.20±13.00	63.00±25.00	7	1.85	0.03*	44.83
Gum Tree	90.30±12.00	85.00±17.00	8	0.26	0.80	5.87
West African pepper	49.50±2.50	27.83±3.10	9	5.51	<0.01*	43.77
CFU/ml (10^{-2})						
Control	18.00±1.00	12.50±0.50	4	0.86	0.84	30.56
African Nutmeg	21.67±1.80	4.50±0.85	7	8.55	<0.01*	79.23
Gum Tree	37.50±2.40	30.30±5.80	3	0.29	0.78	19.20
African pepper	26.17±2.70	12.83±2.90	9	3.35	0.01*	50.97
West African pepper	13.00±1.10	6.67±1.50	9	3.32	<0.01*	48.69

*indicates statistical significance at 95%

Table 2: presents the variation in microbial load in treated and smoked *Synodontis schall*. The result showed that there was no significant difference in the control before and after storage but the lowest microbial load was seen in African nutmeg treated fish, Gum tree treated fish and West African pepper treated fish followed the same trend showing no significant difference with lowest microbial load of ($68.7x10^0$), ($56.50x10^0$) and ($35.80x10^0$) respectively. However, African nutmeg showed a significant difference before and after storage with lowest microbial load of ($22.00x10^{-2}$). In the same vane, CFU/ml (10^{-2}) depicts that apart from African pepper treated fish no other spice treated fish showed a significant difference before and after storage but low microbial load were recorded in African pepper ($3.33x10^{-2}$), African nutmeg ($16.83x10^{-2}$), Gum tree ($12.80x10^2$), West African pepper ($5.00x10^{-2}$).

Table 2: Microbial Evaluation of *Synodontis schall* before and after storage

Microbial Parameter/ Biopreservatives	Time of Storage Before Storage	After Storage	Df	T-Value	P-Value	Percentage (%) effect
CFU/ml (10^0)						
Control	69.50±1.40	24.50±1.40	3	2.27	0.26	64.75
African Nutmeg	96.00±2.90	68.7±4.30	4	0.94	0.40	28.44
African pepper	87.30±6.80	22.00±4.80	7	7.81	<0.01*	74.80
Gum Tree	99.00±8.70	56.50±3.30	3	1.26	0.28	42.93
West African pepper	49.67±4.00	35.80±8.80	5	1.43	0.21	27.92
CFU/ml (10^{-2})						
Control	18.00±1.00	9.50±6.50	1	1.29	0.41	47.22
African Nutmeg	21.90±1.50	16.83±1.80	4	0.32	0.76	23.15

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African pepper	20.83±3.00	3.33±0.88	6	5.67	<0.01*	84.01
Gum Tree	14.67±0.84	12.80±4.10	5	0.44	0.67	12.75
West African pepper	9.33±0.88	5.00±3.00	1	1.39	0.39	46.41

*indicates statistical significance at 95%

4. DISCUSSION

The result of the microbial load in this study shows an improved end product and a prolonged shelf- life in *Clarias gariepinus* and *Synodontis schall*, this may not be unconnected to the fact that the spice treatment and the smoking process seem sufficient to have destroyed microbes. The reduction of microbes in some spices as seen under ambient condition observed in this study indicates the effectiveness of spices as anti-microbial agents and resulted in the extended shelf-life of spice treated samples; in agreement with Alfonso *et al.*, (2017) who reported that the addition of lemon essential oils (EO) micro-emulsions retarded the growth of *Enterobacteriaceae*, *Staphylococci*, and rod-shaped lactic acid bacteria in salted sardines. Likewise, hot smoked tilapia (*Oreochromis niloticus*) treated using EOs (extracts of ginger, garlic, clove) had significantly lowered total viable, total psychotropic, lactic acid bacteria count for 7 weeks storage period (Tairu *et al.*, 2019). Shelf life and quality of fish could be extended, thereby reducing the majority of post-harvest losses by the use of spices (Erkmen and Bozoglu, 2016). Tairu *et al*., (2019) observed that the flesh of ginger-treated fish suggests that ginger contains chemicals that prevent the hatching of eggs that were deposited on the fish prior to treatment with ginger paste, preventing spoilage and extending the shelf life of fish, this is in line with the findings of this study which shows that *Clarias gariepinus* and *synodontis schall* treated with *Piper guineense, Monodora myristica* and *Xylophia aethiopica* shows that the bacteria was reduced after 4 months which also agrees with (Zhan *et al*., 2020) who aserted that with continuous research, the antimicrobial activities of spices, herbs, and essential oils have been well known For example, aniseed, black cumin, black pepper, cardamom, and turmeric can inhibit the growth of aspergillus and the synthesis of aflatoxin as also reported by (Farinha *et al*.,2017) This also agrees with the findings of Tamfu *et al*., (2020) and Orji *et al.*,(2023) who aserted that P. *guineense*, M. *myristica* and X. *aethiopica* have antimicrobial effects especially X. *aethiopica* and M. *myristica* and may offer useful alternatives in aquaculture settings in terms of fish preservation and extending the shelf life of fish. This study is particularly relevant at the time where the world clamours for organic consumables to replace chemical treatments of food. The study has established that the above-mentioned spices can alternatively keep fish safe for a period of 4 months.

5. CONCLUSION

It was concluded that the reduction of the microbial activities and microbes on the fish after 4 months signify the inhibitory properties of spices as reported by several authors this means that P. *guineense*, M. *myristica*, X. *aethiopica* and M. myristica have antimicrobial effects and can be used to minimize the activities of bacteria causing fish spoilage for a period of 4 months or for longer times using spices as preservatives.

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