Original Research Article

ANTIFUNGAL AND PRESERVATIVE EFFECT OF DIFFERENT SPECIES OF *AFRAMOMUM* (K. SCHUM) ON FUNGI ISOLATED FROM RAW MEAT AND FISH.

ABSTRACT

*Aframomum*species (*Aframomumdanielli, Aframomummelegueta and Aframomumsceptrum*) are used traditionally as medicine and food preservatives. Synthetic preservatives have been reported to be carcinogenic; hence, the continuous search for natural preservative. This study was designed to validate the efficacy of the three named *Aframomum* species as preservative against fungi that causes spoilage in raw fish and meat.The methanolic extracts of the samples were screened against *Aspergillustamarii, Aspergillusfumigatus, Aspergillusochraceus*and *Trichoderma*sp using pour plate technique. The fungi was isolated from the raw fish and meat by the method of serial dilution then pour plated into Potatoes Dextro Agar (PDA) incubated at 37^{o}c for 7 days. The fungi observed was sub cultured to get a pure culture.The three samples showed significant antifungal activities against *Aspergillustamarii, Aspergillusfumigatus, Aspergillusochraceus*and *Trichoderma*sp at 25%, 50% and 75% concentrations.The significant antifungal activities displayed by extract of these samples could be attributed to their phytochemical and nutritional components of the samples as well as their antioxidant activity. The three samples could be valuable natural preservatives with additional therapeutic potential.

Keywords*: Aframomum sp*, Peservatives, Treatments, Antioxidant activity, Antifungal.

INTRODUCTION

In tropical Africa, and indeed Nigeria, *aframomum*species is cultivated mainly for its use in ethnomedicine than as a spice (Norton, 2004). The seeds of this indigenous spice have also been found to contain phytochemicals, which is use as medicine,preservatives for herbal medicine and food.(Fasoyiro and Adegoke, 2007).Food spoilage is a metabolic process that causes foods to be undesirable or unacceptable for human consumption due to changes in sensory characteristics. Spoiled foods may be safe to eat, that is they may not cause illness because there are no pathogens or a toxin present, but changes in texture, smell, taste, or appearance cause them to be rejected (Burkepile *et al*., 2006).Preservative may be added to prevent the growth of fungi and bacteria, nearly all food products have food preservatives. Preservatives are substances that commonly added to various foods and pharmaceutical products to prolong their shelf life. The addition of preservatives to food products, especially those with high moisture content, is essential for avoiding alteration and decomposition by microbes during storage.

In recent years have researchers seriously considered the physical impact of these additives over the long term use (walker *et al*., 2007). Consumers are increasingly awae of the risk posed by synthetic antioxidants due to their high volatility and instability at elevated temperatures. As a result of this, focus has been shifted to the use of natural antioxidants in food preservation which has led to a search for novel antimicrobial compounds from natural sources (Odukoya *et al*., 2005). Therefore, there is a need for natural preservative that is cheap and readily available

This work examined the effect of three selected*Aframomum* species*(A.melegueta*, *A.danielli*, *A.sceptrum*) on fungal isolates of fresh fish and meat. This was with a view to provide scientific information on their use as natural preservatives, as well as compared the potency of the three plant samples as antifungal agents.

MATERIALS AND METHODS

Collection, identification and preparation of plant samples.

The indigenous names of the three species were used in their purchase in Oja Oba market in Ibadan Oyo state, Nigeria. The species was identified at the Department of Botany herbarium at the University of Ibadan. Fresh meat (beef) and fish (Markrel, scientific name:*Scomberjaponicus*) were purchased from Bodija market in Ibadan, Oyo state, Ibadan.

Isolation of fungi from fish and meat

Fungi used for this study were isolated from the raw fish and raw meat (beef) that were purchased from the market in Ibadan (Bodija market).Ten mills (10mls) of sterile distilled water was added to the fish and meat and was put in two different beakers, then left for about 30 minutes. One mills and 3mls of each of the solution was pipetted into the petri dishes respectively and potato Dextrose Agar (PDA) which was prepared, autoclaved at 121^{o}C for 15 minutes. It was later allowed to slightly cool before the addition of two drops of latic acid was added to prevent bacterial growth contamination and the agar was poured aseptically into the petri dish beside a Bunsen flame in an inoculating chamber, plates was incubated at 25^{o}C for 5-7days in an incubator and observed daily for fungal growth. The pure of culture of organism isolated from raw meat and fish was identified by pathologist as; *Aspergillus tamarii, Aspergillusfumigatus, Aspergillus ochraceus* and *Trichoderma* sp

Preparation of extracts

The seeds of the *Aframomum* species that were bought in Oja Oba, were air dried for 2weeks and then grinded. Methanolic extracts of the fruits were obtained by weighing the grinded fruit part.200g, 119g and 105g (separately) of the *A. danielli, A. sceptrum, A. melegueta* respectively and soaked in 650mls of methanol. It was left for 2weeks and it was shaken daily using a shaker. The extract was

filtered using No.1 Whatman filter paper, the filtrate was then concentrated to dryness using a rotary evaporator at $40^{O}C$ under a reduced pressure. The paste obtained was used as stock material for further investigation and it is kept at $4^{O}C$ in a refrigerator till when ready to use in accordance with (Abukakar *et al*., 2008).

Antifungal Screening Test

The test was carried out on PDA agar plates using the pour plate technique.25%, 50% and 75% concentration of the dissolved extract was dispensed separately into petri dishes and mixed with PDA. They were inoculated at $28^{o}C$ after which the radius of the growth of the test fungi was measured. Measurement of radius (R1 and R2) was made daily for 10days. For each concentration of the extract test was repeated in three replicates.

Statistical Analysis

Data collected were categorized and analysed using Costat statistical software and the homogeneity of means was done using Duncan multiple Range Test (DMRT). Data were represented as mean $\pm$ standard deviation.

RESULTS

Table 1 : Effect of treatment with 25% formulation of extracts of samples on daily growth of fungal isolates

25% Botanical Formulation	**Radial Mycelial Growth (cm)**

Treatment	Fungal Specimen	Day 2	Day 4	Day 6	Day 8
A. danielli	*Aspergillusfumigatus*	0.00 ± 0.00^{g}	0.78 ± 0.35^{ef}	0.87 ± 0.43^{hij}	$1.33\pm0.28^{g-l}$
Control 1	PDA+*A. fumigatus*+ Di-Ether	0.25 ± 0.00^{f}	1.60 ± 0.00^{c}	1.80 ± 0.00^{de}	$2.45\pm0.00^{c-f}$
Control 2	PDA+*Aspergillusfumigatus*	0.45 ± 0.00^{e}	1.35 ± 0.00^{cd}	1.75 ± 0.00^{def}	$1.95\pm0.00^{c-i}$
A. danielli	*Aspergillustamarii*	0.00 ± 0.00^{g}	1.13 ± 0.54^{de}	1.45 ± 0.35^{efg}	$1.77\pm0.50^{d-k}$
Control 1	PDA+*A. tamarii*+Di-Ether	0.00 ± 0.00^{g}	1.20 ± 0.00^{d}	1.75 ± 0.00^{def}	2.85 ± 0.00^{bcd}
Control 2	PDA+*Aspergillustamarii*	0.55 ± 0.00^{c}	2.30 ± 0.00^{b}	2.30 ± 0.00^{bc}	$2.00\pm0.00^{c-h}$
A. danielli	*Aspergillusochraceus*	0.00 ± 0.00^{g}	0.00 ± 0.00^{j}	0.00 ± 0.00^{n}	0.00 ± 0.00^{n}
Control 1	PDA+*A. ochraceus*+Di-Ether	0.00 ± 0.00^{g}	0.00 ± 0.00^{j}	0.10 ± 0.00^{mn}	0.10 ± 0.00^{n}
Control 2	PDA+*Aspergillusochraceus*	0.95 ± 0.00^{b}	2.05 ± 0.00^{b}	2.50 ± 0.00^{b}	3.05 ± 0.00^{abc}
A. danielli	*Trichoderma*sp	0.00 ± 0.00^{g}	0.00 ± 0.00^{j}	0.00 ± 0.00^{n}	0.22 ± 0.03^{mn}
Control 1	PDA+*Trichoderma*sp+Di-Ether	0.00 ± 0.00^{g}	0.00 ± 0.00^{j}	0.00 ± 0.00^{n}	0.30 ± 0.00^{lmn}
Control 2	PDA+*Trichoderma*sp	1.70 ± 0.00^{a}	3.80 ± 0.00^{a}	4.00 ± 0.00^{a}	4.00 ± 0.00^{a}
A. sceptrum	*Aspergillusfumigatus*	0.00 ± 0.00^{g}	$0.43\pm0.08^{f-i}$	0.98 ± 0.33^{hi}	$1.30\pm0.20^{g-m}$
Control 1	PDA+*A. fumigatus*+Di-Ether	0.00 ± 0.00^{g}	0.25 ± 0.00^{hij}	0.65 ± 0.00^{ijk}	$0.95\pm0.00^{h-n}$
Control 2	PDA+*A. fumigatus*	1.70 ± 0.00^{a}	0.65 ± 0.00^{fg}	$0.80\pm0.00^{h-k}$	$1.00\pm0.00^{h-n}$
A. sceptrum	*Aspergillustamarii*	0.00 ± 0.00^{g}	$0.43\pm0.03^{f-i}$	0.92 ± 0.21^{hij}	$1.25\pm0.10^{h-m}$
Control 1	PDA+*A. tamarii*+Di-Ether	0.50 ± 0.00^{d}	1.45 ± 0.00^{cd}	1.95 ± 0.00^{cd}	$1.95\pm0.00^{c-i}$
Control 2	PDA+*Aspergillustamarii*	0.00 ± 0.00^{g}	0.75 ± 0.00^{f}	1.15 ± 0.00^{gh}	$1.35\pm0.00^{f-l}$
A. sceptrum	*Trichoderma*sp	0.00 ± 0.00^{g}	0.00 ± 0.00^{jj}	0.27 ± 0.08^{lmn}	0.37 ± 0.06^{lmn}
Control 1	PDA+*Trichoderma*sp+Di-Ether	0.00 ± 0.00^{g}	$0.35\pm0.00^{g-j}$	0.60 ± 0.00^{jkl}	$0.90\pm0.00^{h-n}$
Control 2	PDA+*Trichoderma*sp	0.00 ± 0.00^{g}	0.45 ± 0.00^{fghi}	0.65 ± 0.00^{ijk}	$0.90\pm0.00^{h-n}$
A. sceptrum	*Aspergillusochraceus*	0.00 ± 0.00^{g}	0.10 ± 0.00^{ij}	0.1 ± 0.00^{mn}	$0.75\pm0.61^{j-n}$
Control 1	PDA+*A. ochraceus*+Di-Ether	0.00 ± 0.00^{g}	$0.35\pm0.00^{g-j}$	0.55 ± 0.00^{jkl}	3.70 ± 0.00^{ab}
Control 2	PDA+*A. ochraceus*	0.00 ± 0.00^{g}	$0.30\pm0.00^{g-j}$	0.60 ± 0.00^{jkl}	$0.85\pm0.00^{i-n}$
A. melegueta	*Aspergillusfumigatus*	0.00 ± 0.00^{g}	0.48 ± 0.08^{fgh}	0.68 ± 0.08^{ijk}	$0.92\pm0.10^{h-n}$
Control 1	PDA+*A. fumigatus*+Di-Ether	0.00 ± 0.00^{g}	1.30 ± 0.00^{cd}	1.90 ± 0.00^{d}	$1.90\pm0.00^{d-i}$
Control 2	PDA+*A. fumigatus*	0.00 ± 0.00^{g}	1.35 ± 0.00^{cd}	1.75 ± 0.00^{def}	$1.95\pm0.00^{c-i}$

A. melegueta	*Trichoderma*spp	0.00±0.00^{g}	0.00±0.00^{j}	0.00±0.00^{n}	0.20±0.09^{mn}
Control 1	PDA+*Trichoderma*sp+Di-Ether	0.00±0.00^{g}	0.00±0.00^{j}	0.00±0.00^{n}	0.35±0.00^{lmn}
Control 2	PDA+*Trichoderma*sp	0.00±0.00^{g}	2.00±0.00^{b}	2.30±0.00^{bc}	2.5-*0±0.00^{cde}
A. melegueta	*Aspergillustamarii*	0.00±0.00^{g}	0.27±0.03^{hij}	0.45±0.00^{klm}	0.70±0.00^{k-n}
Control 1	PDA+*A. tamarii*+Di-Ether	0.00±0.00^{g}	1.15±0.00^{d}	1.40±0.00^{fg}	1.85±0.00^{d-j}
Control 2	PDA+*A. tamarii*	0.00±0.00^{g}	2.20±0.00^{b}	2.45±0.00^{b}	2.40±0.00^{c-g}
A. melegueta	*Aspergillusochraceus*	0.00±0.00^{g}	0.00±0.00^{j}	0.25±0.13^{lmn}	1.40±1.83^{e-l}
Control 1	PDA+*A. ochraceus*+Di-Ether	0.00±0.00^{g}	0.00±0.00^{j}	0.25±0.00^{lmn}	0.40±0.00^{lmn}
Control 2	PDA+*A. ochraceus*	0.00±0.00^{g}	0.55±0.00^{fgh}	0.75±0.00^{ijk}	1.00±0.00^{h-n}

Means are based on triplicate reading

Table 1 shows the activities of 25% concentration of *aframomum* species in which the treatment and days of treatment had significant effect on the growth of the organism inhibition. *Aspergillusfumigatus* was significantly ($p \leq 0.05$) inhibited by*Aframomumdanielli* from day 2 till day 6 (Day 2[0.00±0.00], Day 4 [0.78± 0.35] and Day 6 [0.87± 0.43]) when compared to the control set up for *A. fumigatus*for the di-ethylether extract.There was no significant $p \leq 0.05$) impact of *A. danielli*at 25 % concencentration on the radial mycelial growth of *Aspergillustamarii*, *Aspergillusochraceus and Trichoderma*sp. The di-ethyletherextract of *Aframomumsceptrum*had no significant ($p \leq 0.05$) impact on the radial mycelial growth of (*Aspergillustamarii,Aspergillusochraceus and Trichoderma*sp, *A. fumigatus*). *Aframomummelegueta*had significant ($p \leq 0.05$) effect on the radial mycelial growth of *A.fumigatus* at day 4 (0.48±0.08) and 6 (0.68±0.08) only.*Aspergillusochraceus, A. tamarii*, and *Trichodermasp* were not significantly inhibited by the plant compared to that of the control.

Table2 : Effect of treatment with 50 % formulation of extracts of samples on daily growth of fungal isolates

50% Botanical Formulation		**Radial Mycelial Growth (cm)**			
Treatment	**Fungal Specimen**	**Day 2**	**Day 4**	**Day 6**	**Day 8**
A. danielli	*Aspergillusfumigatus*	0.00 ± 0.00^{g}	0.53 ± 0.03^{jk}	0.82 ± 0.13^{fg}	$1.22\pm0.24^{e-i}$
Control 1	PDA+*A. fumigatus*+ Di-Ether	0.25 ± 0.00^{f}	1.60 ± 0.00^{e}	1.80 ± 0.00^{c}	2.45 ± 0.00^{cde}
Control 2	PDA+*Aspergillusfumigatus*	0.45 ± 0.00^{e}	1.35 ± 0.00^{fg}	1.75 ± 0.00^{c}	$1.95\pm0.00^{c-g}$
A. danielli	*Aspergillustamarii*	0.00 ± 0.00^{g}	0.68 ± 0.25^{ij}	0.90 ± 0.13^{f}	$1.40\pm0.05^{d-h}$
Control 1	PDA+*A. tamarii*+Di-Ether	0.00 ± 0.00^{g}	1.20 ± 0.00^{gh}	1.75 ± 0.00^{c}	2.85 ± 0.00^{abc}
Control 2	PDA+*Aspergillustamarii*	0.55 ± 0.00^{c}	2.30 ± 0.00^{b}	2.30 ± 0.00^{b}	$2.00\pm0.00^{c-g}$
A. danielli	*Aspergillusochraceus*	0.00 ± 0.00^{g}	0.00 ± 0.00^{o}	0.00 ± 0.00^{l}	0.10 ± 0.00^{i}
Control 1	PDA+*A. ochraceus*+Di-Ether	0.00 ± 0.00^{g}	0.00 ± 0.00^{o}	0.10 ± 0.00^{kl}	0.10 ± 0.00^{i}
Control 2	PDA+*Aspergillusochraceus*	0.95 ± 0.00^{b}	2.05 ± 0.00^{cd}	2.50 ± 0.00^{b}	3.05 ± 0.00^{abc}
A. danielli	*Trichoderma*sp	0.00 ± 0.00^{g}	0.00 ± 0.00^{o}	0.00 ± 0.00^{l}	0.17 ± 0.18^{hi}
Control 1	PDA+*Trichoderma*sp+Di-Ether	0.00 ± 0.00^{g}	0.00 ± 0.00^{o}	0.00 ± 0.00^{l}	0.30 ± 0.00^{hi}
Control 2	PDA+*Trichoderma*sp	1.70 ± 0.00^{a}	3.80 ± 0.00^{a}	4.00 ± 0.00^{a}	4.00 ± 0.00^{a}
A. sceptrum	*Aspergillusfumigatus*	0.00 ± 0.00^{g}	0.30 ± 0.09^{lm}	0.88 ± 0.28^{f}	$1.13\pm0.53^{f-i}$
Control 1	PDA+*A. fumigatus*+Di-Ether	0.00 ± 0.00^{g}	0.25 ± 0.00^{mn}	0.65 ± 0.00^{gh}	0.95 ± 0.00^{ghi}
Control 2	PDA+*A. fumigatus*	1.70 ± 0.00^{a}	0.65 ± 0.00^{ij}	0.80 ± 0.00^{fg}	$1.00\pm0.00^{f-i}$
A. sceptrum	*Aspergillustamarii*	0.00 ± 0.00^{g}	0.00 ± 0.00^{o}	0.77 ± 0.23^{fgh}	$1.05\pm0.13^{f-i}$
Control 1	PDA+*A. tamarii*+Di-Ether	0.50 ± 0.00^{d}	1.45 ± 0.00^{ef}	1.95 ± 0.00^{c}	$1.95\pm0.00^{c-g}$
Control 2	PDA+*Aspergillustamarii*	0.00 ± 0.00^{g}	0.75 ± 0.00^{i}	1.15 ± 0.00^{e}	$1.35\pm0.00^{d-h}$
A. sceptrum	*Trichoderma*sp	0.00 ± 0.00^{g}	0.00 ± 0.00^{o}	0.20 ± 0.00^{jkl}	0.25 ± 0.00^{hi}
Control 1	PDA+*Trichoderma*sp+Di-Ether	0.00 ± 0.00^{g}	0.35 ± 0.00^{lm}	0.60 ± 0.00^{ghi}	0.90 ± 0.00^{ghi}

Control 2	PDA+*Trichoderma*sp	0.00±0.00^{g}	0.45±0.00^{kl}	0.65±0.00^{gh}	0.90±0.00^{ghi}
A. sceptrum	*Aspergillusochraceus*	0.00±0.00^{g}	0.10±0.00^{no}	0.10±0.00^{kl}	0.58±0.23^{hi}
Control 1	PDA+*A. ochraceus*+Di-Ether	0.00±0.00^{g}	0.35±0.00^{lm}	0.55±0.00^{hi}	3.70±0.00^{ab}
Control 2	PDA+*A. ochraceus*	0.00±0.00^{g}	0.30±0.00^{lm}	0.60±0.00^{ghi}	0.85±0.00^{ghi}
A. melegueta	*Aspergillusfumigatus*	0.00±0.00^{g}	0.42±0.08^{kl}	0.65±0.05^{gh}	2.20±2.17^{c-f}
Control 1	PDA+*A. fumigatus*+Di-Ether	0.00±0.00^{g}	1.30±0.00^{fgh}	1.90±0.00^{c}	1.90±0.00^{c-g}
Control 2	PDA+*A. fumigatus*	0.00±0.00^{g}	1.35±0.00^{fg}	1.75±0.00^{c}	1.95±0.00^{c-g}
A. melegueta	*Trichoderma*spp	0.00±0.00^{g}	0.00±0.00^{o}	0.00±0.00^{l}	0.25±0.00^{hi}
Control 1	PDA+*Trichoderma*sp+Di-Ether	0.00±0.00^{g}	0.00±0.00^{o}	0.00±0.00^{l}	0.35±0.00^{hi}
Control 2	PDA+*Trichoderma*sp	0.00±0.00^{g}	2.00±0.00^{d}	2.30±0.00^{b}	2.50±0.00^{bcd}
A. melegueta	*Aspergillustamarii*	0.00±0.00^{g}	0.20±0.05^{mn}	0.40±0.00^{ij}	0.60±0.05^{hi}
Control 1	PDA+*A. tamarii*+Di-Ether	0.00±0.00^{g}	1.15±0.00^{h}	1.40±0.00^{d}	1.85±0.00^{c-g}
Control 2	PDA+*A. tamarii*	0.00±0.00^{g}	2.20±0.00^{bc}	2.45±0.00^{b}	2.40±0.00^{cde}
A. melegueta	*Aspergillusochraceus*	0.00±0.00^{g}	0.02±0.03^{o}	0.12±0.10^{kl}	0.28±0.06^{hi}
Control 1	PDA+*A. ochraceus*+Di-Ether	0.00±0.00^{g}	0.00±0.00^{o}	0.25±0.00^{jk}	0.40±0.00^{hi}
Control 2	PDA+*A. ochraceus*	0.00±0.00^{g}	0.55±0.00^{jk}	0.75±0.00^{fgh}	1.00±0.00^{f-i}

Means are based on triplicate reading

Table 2 shows the bioassay of 50% formulation of *Aframomum* species in which *Aframomumdanielli* at 50% concentration significantly inhibited the growth of *Aspergillusfumigatus* from day 2 till day 6 (Day 2[0.00±0.00], Day 4 [0.53±0.03], and Day 6 [0.82±0.1]).The radial mycelial growth of *Aspergillustamarii*, *Aspergillusochraceus* and *Trichoderma*sp was not significantly ($p \leq 0.05$) inhibited by *A. danielli* at 50% concentration compared to the di-ethylether solvent used as control. *Aframomumsceptrum* at 50% concentration significantly ($p \leq 0.05$) inhibited the radial mycelial growth of *Aspergillustamarii* from Day 4 (0.00 ± 0.00), and Day 6 (0.77 ± 0.23) .*Aspergillusochraceus* and *Trichoderma*sp

and *Aspergillusfumigatus*were not affected by extract of *Aframomumsceptrum*. *Aframomummelegueta* had significant effect on the radial mycelial growth of *A. tamarii* at day 4 (0.20±0.05) and day 6 (0.40±0.00) while *Aspergillusochraceus*, *A. fumigatus*, and *Trichodermasp* were not significantly inhibited.*Aspergillustamarii* and *Aspergillusfumigatus* was not significantly inhibited by the extract of *Aframomumsceptrum* at $p \leq 0.05$.

Aframomummelegueta had significant effect on the radial mycelial growth of *A. tamarii*at day 4 (0.42±0.14) and 8 (0.90±0.22).

Table3 : Effect of treatment with 75 % formulation of extracts of samples on daily growth of fungal isolates

75% Botanical Formulation		**Radial Mycelial Growth (cm)**			
Treatment	**Fungal Specimen**	**Day 2**	**Day 4**	**Day 6**	**Day 8**
A. danielli	*Aspergillusfumigatus*	0.00 ± 0.00^{g}	0.43 ± 0.08^{kl}	$0.55\pm0.09^{e-h}$	1.00 ± 0.23^{fg}
Control 1	PDA+*A. fumigatus*+ Di-Ether	0.25 ± 0.00^{f}	1.60 ± 0.00^{e}	1.80 ± 0.00^{bc}	2.45 ± 0.00^{d}
Control 2	PDA+*Aspergillusfumigatus*	0.45 ± 0.00^{e}	1.35 ± 0.00^{fg}	1.75 ± 0.00^{bcd}	1.95 ± 0.00^{e}
A. danielli	*Aspergillustamarii*	0.00 ± 0.00^{g}	0.80 ± 0.15^{i}	2.38 ± 1.53^{b}	1.82 ± 0.16^{e}
Control 1	PDA+*A. tamarii*+Di-Ether	0.00 ± 0.00^{g}	1.20 ± 0.00^{gh}	1.75 ± 0.00^{bcd}	2.85 ± 0.00^{bc}
Control 2	PDA+*Aspergillustamarii*	0.55 ± 0.00^{c}	2.30 ± 0.00^{b}	2.30 ± 0.00^{b}	2.00 ± 0.00^{e}
A. danielli	*Aspergillusochraceus*	0.00 ± 0.00^{g}	0.00 ± 0.00^{o}	0.00 ± 0.00^{h}	0.10 ± 0.00^{hi}
Control 1	PDA+*A. ochraceus*+Di-Ether	0.00 ± 0.00^{g}	0.00 ± 0.00^{o}	0.10 ± 0.00^{gh}	0.10 ± 0.00^{hi}
Control 2	PDA+*Aspergillusochraceus*	0.95 ± 0.00^{b}	2.05 ± 0.00^{cd}	2.50 ± 0.00^{b}	3.05 ± 0.00^{b}
A. danielli	*Trichoderma*sp	0.00 ± 0.00^{g}	0.00 ± 0.00^{o}	0.00 ± 0.00^{h}	0.00 ± 0.00^{i}
Control 1	PDA+*Trichoderma*sp+Di-Ether	0.00 ± 0.00^{g}	0.00 ± 0.00^{o}	0.00 ± 0.00^{h}	0.30 ± 0.00^{hi}
Control 2	PDA+*Trichoderma*sp	1.70 ± 0.00^{a}	3.80 ± 0.00^{a}	4.00 ± 0.00^{a}	4.00 ± 0.00^{a}
A. sceptrum	*Aspergillusfumigatus*	0.00 ± 0.00^{g}	0.30 ± 0.09^{lm}	$0.88\pm0.28^{d-g}$	1.13 ± 0.53^{fg}
Control 1	PDA+*A. fumigatus*+Di-Ether	0.00 ± 0.00^{g}	0.25 ± 0.00^{mn}	$0.65\pm0.00^{e-h}$	0.95 ± 0.00^{g}

Control 2	PDA+*A. fumigatus*	1.70±0.00^{a}	0.65±0.00^{ij}	0.80±0.00^{e-h}	1.00±0.00^{fg}
A. sceptrum	*Aspergillustamarii*	0.00±0.00^{g}	0.40±0.15^{klm}	0.87±0.19^{e-h}	1.17±0.20^{fg}
Control 1	PDA+*A. tamarii*+Di-Ether	0.50±0.00^{d}	1.45±0.00^{ef}	1.95±0.00^{bc}	1.95±0.00^{e}
Control 2	PDA+*Aspergillustamarii*	0.00±0.00^{g}	0.75±0.00^{i}	1.15±0.00^{c-f}	1.35±0.00^{f}
A. sceptrum	*Trichoderma*sp	0.00±0.00^{g}	0.10±0.00^{no}	0.20±0.00^{gh}	0.25±0.00^{hi}
Control 1	PDA+*Trichoderma*sp+Di-Ether	0.00±0.00^{g}	0.35±0.00^{lm}	0.60±0.00^{e-h}	0.90±0.00^{g}
Control 2	PDA+*Trichoderma*sp	0.00±0.00^{g}	0.45±0.00^{kl}	0.65±0.00^{e-h}	0.90±0.00^{g}
A. sceptrum	*Aspergillusochraceus*	0.00±0.00^{g}	0.10±0.00^{no}	0.32±0.06^{fgh}	0.40±0.05^{h}
Control 1	PDA+*A. ochraceus*+Di-Ether	0.00±0.00^{g}	0.35±0.00^{lm}	0.55±0.00^{e-h}	3.70±0.00^{a}
Control 2	PDA+*A. ochraceus*	0.00±0.00^{g}	0.30±0.00^{lm}	0.60±0.00^{e-h}	0.85±0.00^{g}
A. melegueta	*Aspergillusfumigatus*	0.00±0.00^{g}	0.53±0.13^{jk}	0.67±0.08^{e-h}	0.88±0.08^{g}
Control 1	PDA+*A. fumigatus*+Di-Ether	0.00±0.00^{g}	1.30±0.00^{fgh}	1.90±0.00^{bc}	1.90±0.00^{e}
Control 2	PDA+*A. fumigatus*	0.00±0.00^{g}	1.35±0.00^{fg}	1.75±0.00^{bcd}	1.95±0.00^{e}
A. melegueta	*Trichoderma*spp	0.00±0.00^{g}	0.00±0.00^{o}	0.00±0.00^{h}	0.10±0.00^{hi}
Control 1	PDA+*Trichoderma*sp+Di-Ether	0.00±0.00^{g}	0.00±0.00^{o}	0.00±0.00^{h}	0.35±0.00^{hi}
Control 2	PDA+*Trichoderma*sp	0.00±0.00^{g}	2.00±0.00^{d}	2.30±0.00^{b}	2.50±0.00^{cd}
A. melegueta	*Aspergillustamarii*	0.00±0.00^{g}	0.42±0.14^{klm}	0.65±0.22^{e-h}	0.90±0.22^{g}
Control 1	PDA+*A. tamarii*+Di-Ether	0.00±0.00^{g}	1.15±0.00^{h}	1.40±0.00^{cde}	1.85±0.00^{e}
Control 2	PDA+*A. tamarii*	0.00±0.00^{g}	2.20±0.00^{bc}	2.45±0.00^{b}	2.40±0.00^{d}
A. melegueta	*Aspergillusochraceus*	0.00±0.00^{g}	0.05±0.05^{o}	0.17±0.16^{gh}	0.30±0.10^{hi}
Control 1	PDA+*A. ochraceus*+Di-Ether	0.00±0.00^{g}	0.00±0.00^{o}	0.25±0.00^{gh}	0.40±0.00^{h}
Control 2	PDA+*A. ochraceus*	0.00±0.00^{g}	0.55±0.00^{jk}	0.75±0.00^{e-h}	1.00±0.00^{fg}

Means are based on triplicate reading

Table 3 showed that *Aspergillusfumigatus* was significantly inhibited by 75% plant extract formulation of *Aframomumdanielli* (Day 2 [0.00±0.00], Day 4 [0.43±0.08], Day 6 [0.55±0.09 and Day 8 [1.00±0.23]. The radial mycelial growth of *Aspergillustamarii* was significantly impacted by the extract of *A. danielli* at 75% concentration at day 4 (0.80±0.15, other pathogens

were significantly unaffected by the extract when compared to either of the control. *Aframomumsceptrum* significantly inhibited the radial mycelial growth of *Aspergillustamarii* Day 4 (0.40±0.15) when compared to the control while *Trichodermasp* was affected by 75% concentration of *A. sceptrum* at day 8 (0.25±0.00). *Aframomummelegueta* had significant effect on the radial mycelial growth of *A. tamarii*at day 4 (0.42±0.14) and 8 (0.90±0.22).

DISCUSSION

In this research, *Aspergillustamarii,Trichodermasp, Aspergillusfumigatus and Aspergillusochraceus*were isolated from raw meat and fish which is similar to the findings ofAyeloja*et al.,* 2013.For ages*aframomumsp* has been used traditionally for the treatment of diseases. The extracts of the seeds have been used for years in the treatment of infectious diseases as well as in treating wounds and prevention of infections.Alligator pepper extract was used by Okigbo*etal*.,2006 in the control of *Fusariumoxsporum* and *A.tamari*. Iwu 1986 reported that *A.melegueta* has antimicrobial and antifungal effects. It is believed that the methanolic extracts of *Afranmomumsp* are more fungitoxic than water extracts, these active principles were probably extracted by methanol.This agrees with Okigbo*etal*., 2006 who observed that factors like the type of extracting solvent and age of the plant could influence the active principle present in the plant. The plant extracts differed significantly in their potential to inhibit the growth of these fungal pathogens. *A.tamarii* was not inhibited at the lower concentration by the extract of *A.danielli*.*A.danielli* inhibited the growth of *A.fumigatus* and some spoilage pathogens. This result was similar to the work reported by Adegoke and Skura 2000.Extract of *A.melegueta* appears to pose stronger antifungal properties against the mycelia growth of *A.tamarii* at higher concentaration The inhibitory effect of the plant extract at lower concentration showed that only

A.fumigatus was inhibited, at this same concentration *A.melegueta* had significant difference on *A.fumigatus* while other pathogen were unaffected. *A.fumigatus and A.tamarii* was the most inhibited by the plant extracts, while *A.ocheraues* and *trichodermasp*was the least inhibited. However, the inhibition of the mycelia growth of all the tested pathogen took a similar trend in two of the plant extract except for *A. sceptrum*. Increase in antifungal activity was observed with the corresponding increase in the concentration of the plant extract, this agrees with the work of Amadioha, (2002).

Ilondu*et al.,* (2000) reported that some plant contain phenolic substance and essential oil, which are inhibitory to microorganism, the presence of these compounds in these extract have been reported to be responsible for their antifungal properties (Ahmed and stoll 2000). These antifungal properties control various pests including fungi while the extract of *A.melegueta* and *A.danielli* is specially valued for their effectiveness against fungi (Ahmed and stoll 2000).

The plant extracts differed significantly in their potential to inhibit the growth of this fungal pathogen. It can be noted that the concentration of the extract at the highest concentration had significant effect on the mycelia growth of these pathogens expect for *A. ochraceus* which was not inhibited by the extract. It is noteworthy that *A. melegueta* was more inhibitory than all others; the inhibitory potency of the plant extracts may be attributed to the phytochemical compound like alkaloids, flavonoids and saponnin as reported byAdegoke and Skura 2000.

Szabo*et al*., 2010 reported the high potency of the plant extract containing the same bioactive compound could be used to control fungal pathogen in food. The greater efficiency of *A.melegueta* may be due to its high alkaloids (Chiejina and Ukeh, 2002), since alkaloids are ranked the most efficient therapeutically significant plant substance.

CONCLUSION

The seeds of *Aframomum* has nutritional values and hence it's therapeutic and ethnomedicinal uses. In which natural plant products which have shown to be useful in protecting food against fungal infection and consequent mycotoxin production were shown to retard fungal growth in this study. The significant antioxidant of the seeds is an indication that it could be useful in the management of diseases due to oxidative stress, and obesity. If these plants are used in the storage of fish and meat, they could reduce its loss in storage and also the consumption of mycotoxin-contaminated foods especially in populations where fish and meat constitute a major portion of the diet. The use of natural plant products in preserving could also eliminate the problem of chemical poisoning that could arise from use of synthetic chemicals in the storage of fish and meat. *Aframomumdanielli* has been over exploited; hence there is a need to consider the other two species as alternatives to *Aframomumdanielli* as herbal remedy and preservatives

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