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Full Length Research Paper

Antimicrobial activities of extract of four local spices on bacteria isolated from raw meat sold in Abraka, Delta State, Nigeria

Nwafor, O. Emmanuel

Department of Microbiology, Delta State University, Abraka, Nigeria
Email: obiomanwafor@yahoo.com

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Antimicrobial effect ofethanolic extracts of nutmeg *(Myristica fragrans*), African Ethiopian pepper, (*Xylopia aetiopica*) ginger (*Zingber officinale*) and garlic on micro organisms isolated from raw meat was investigated. The extracts were applied at concentrations of 5.00, 10.00, 15.00 and 20.00mg/mL. The microorganisms tested were *Escherichia coli, Pseudomonas pneumoniae, Bacillus cereus, Klebsiella aerogenes, Staphylococcus aureus, Salmonella typhi* and *Enterobacter* sp. Effect of the spices' extracts on the growth of the organisms as measured by zones of inhibition, showed varying degrees of growth inhibition. *Klebsiella aerogenes* showed the least sensitivity while *Salmonella typhi* showed the highest sensitivity with nutmeg. *E. coli* was more sensitive to ginger. The minimum inhibitory concentration (MIC) was 15.00mg/mL. for all extracts on the microorganisms except that of nutmeg on *P. pneumoniae* and *Salmonella typhi*, African Ethiopian pepper on *Staphylococcus aureus*, ginger on *E. coli* and garlic on *B.cereus* with MIC value of 10.00g/mL. The minimum bactericidal concentrations range from 15.00mg/mL to 20.00mg/mL while the highest percentage inhibition was recorded with African Ethiopian pepper on. The study has indicated the possibility of control of meat deterioration that may be due these microorganisms.

Keywords: Antimicrobial, activities, extracts, spices, bacteria, meat.

INTRODUCTION

Meat is animal tissue, mostly skeletal muscles and associated fat used as food (Hammer,1997). Recent definition has however included other species such as fish, shellfish, poultry and exotic ones as frogs and alligator (Moddler ,2000*; Iroha et al*; 2011). The rich nutrient matrix of meat is now the first choice of animal protein for many people all over the world (Heinz and Hautzinger 2007).

There is no accurate record of the quantity of meat consumed in Nigeria. It is however estimated that in Canada by 2008 the consumption of meat was 36.60Kgper capita. The breakdown of which is beef and veel 12.80 Kg per capita, chicken 11.20 Kg per capita, turkey 2.40 Kg per capita, pork 9.70 Kg per capita and lamb 0.50 Kg per capita (SC,2009). In the USA, in 2007, consumption was put at 101Kg per capita (THSUS,2010). With the increasing consumption of meat worldwide, the annual per capita consumption is expected to reach 37Kg per capita by 2030(Heinz and Hautzinger, 2007).

Intact tissues of animals are sterile but when these animals are slaughtered they get contaminated by microorganisms characteristic of the environment and the

Table 1. Frequency distribution of bacterial isolates in different meat samples.

Bacterial isolates	chicken	beef	pork	goat meat
E. coli	05	20	25	15
P.pneumoniae	-	-	06	03
B.cereus	02	-	02	-
K.aerogenes	-	04	03	-
Staph.aureus	-	03	06	04
S. typhi	-	02	04	04
*Enterobacter*sp	04	10	15	10

Table 2. Effect of spices' extracts on growth of bacteria isolated from meat.

Extract	Bacterial isolates Zone of inhibition (mm)			
	Nutmeg	Pepper	Ginger	Garlic
E.coli	9.00± 0.31	6.03± 0.32	10.50± 0.30	9.50±0 0.20
P. pneumoniae	10.60± 0.33	7.40± 0.30	10.80± 0.22	10.300.±0.20
B. cereus	8.40± 0.31	7.50± 0.32	9.30± 0.30	8.40± 0.22
K.aerogenes	6.40± 0.31	5.00± 0.29	4.20± 0.32	7.20± 0.33
Staph.aureus	9.10± 0.40	6.10± 0.32	9.00 ±0.21	8.90± 0.20
S. typhi	12.00 ±0.30	10.50± 0.33	9.60± 0.41	9.00± 0.32
Enterobacter sp.	8.00 ±0.33	5.40± 0.31	9.70± 0.33	9.00 ±0.32

implements. This happens despite washing of animals before slaughter, various treatments to clean car cases during processing and other programmers' to keep the environment clean (Sperber and Doyle 2009). They therefore lead to the contamination and spoilage of significant portion of the meat Some of the microorganisms are pathogenic while others are spoilage. Raw meat has been reported to be responsible for a significant number of cases of human food poisoning (Geomaras *et al*; 1995). Some of the bacteria implicated include species of *Bacillus, Lactobacillus, Escherichia, Clostridium, Psudomonas, Staphylococcus, Micrococcus and Enterococci (*Zweifer *et al;* 2008*).*

There is therefore the need for proper preservation particularly when meat has to be transported through long distances. Traditional methods of meat preservation include drying, smoking, curing with salt, chilling, fermentation and canning. These methods in most cases do not retain the freshness of meat. The use of chemical preservatives which was adopted (Zhau *et al*; 2010)is equally being discouraged due to their side effects such as toxicity to consumers and bioaccumulation, hence the need for simple and inexpensive safe methods of preserving fresh meat.

Spices are usually added to foods including meat to improve their taste and flavour (Nakatani 1994). There are reports on their antimicrobial activities (Ejechi and Akpomedaye, 2002; Akujobi *et al;* 2006). This study was carried out to investigate the effect of extracts of some local spices against micro organisms involved in meat spoilage. It is expected to provide information on their use for achieving meat stability at ambient temperature.

MATERIALS AND METHODS

Fresh meat samples were bought from Abraka market. There were a total of 200 meat samples made up of beef (n=50), chicken (n=50), pork (n=50) and goat meat (n-50). They were each collected in different sterile polythene bags and transported to the Department of Microbiology laboratory of Delta State University, for processing within 6hs of slaughter.

A portion (25 Kg) of each sample was placed into separate sterile stomacher bay containing 225ml of 0.10% sterile peptone water and pummeled with a mix 1 mixer. Samples were then serially diluted in 0.10% sterile peptone water for microbiological analysis to $10^{-5.}$

MICROBIOLOGICAL ANALYSIS

One milliliter (1mL) from a suitable dilution from the various test tubes was inoculated in triplicate pre-poured and dried nutrient agar (Oxoid) and incubated at 37^0C for 24h. Coli form bacteria were determined by plating on MacConkey agar (Oxoid) and incubating at 37^0C for 24h. Microbial isolates were identified using colonial, morphological and biochemical characteristics (Brown 2005, Vanderzannt and Split to easer, 1992). Morphological features used were Gram reaction, presence/position of spores and motility (Harrigan and Mcance, 1976) while the battery of biochemical tests

Table 3. Minimum Inhibitory concentration of spices' extracts (mg/mL

Bacterial isolates	Extracts			
	Garlic	Nutmeg	Pepper	Ginger
E. coli15.00	15.00	10.00	10.00	
P. pnemoniae	10 .00	15.00	15.00	15.00
B. cereus	10.00	15.00	10.00	15.00
K. aerogenes	15.00	15.00	15.00	15.00
Staph. aureus	15.00	10.00	15.00	15.00
S, typhi10.00	15.00	15.00	15.00	
Enterobactersp	15.00	15.00	15.00	15.00

Table 4. Minimum bactericidal concentration of extracts against bacterial isolates (mg/mL)

Extracts	Bacterial isolates						
E.coli	*P. peumoniae*	*B. cereus*	*K. aerogenes*	*S.aureus*	*S. typhi*	*Enterobactersp*	
Nutmeg	20.00	15.00	20.00	15.00	15.00	15.00	15.00
Pepper	15.00	20.00	20.00	15.00	20.00	20.00	20.00
Ginger	20.00	20.00	20.00	15.00	20.00	20.00	20.00
Garlic	20.00	20.00	20.00	15.00	20.00	20.00	20.00

carried out were catalase, oxidase, urease, coagulase, indole, methyl red, Voges-Proskauer, citrate (IMViC) and sugar fermentation(Sneath *et al*; 1986). Identified isolates were maintained in nutrient agar slants at 4^0C. until when required for further experiments.

Preparation of Spices

The spices, nutmeg (*Myristica fragrans*), African Ethiopian pepper (*Xylopia aetiopica*), ginger (*Zingberofficinale*) and garlic were dried and milled separately using National electric powered dry blender (National Electric Company, Singapore) to powdered form. Extracts were then obtained by soaking 30g of each powder in 100ml of ethanol in 250ml conical flask, properly stopper and covered with aluminum foil. The flasks were left at room temperature for 72h. Thereafter, the content of each flask was filtered using what man No 1 filter paper and filtrate collected in different flasks. The filtrates containing extracts were each concentrated on water bath at 80^0C to a white substance giving yields of 6.50g, 8.00g, 8.90g and 8.40g for nut meg, African Ethiopian pepper, ginger and garlic respectively. They were stored at 4^0C for further use.

Determination of Antimicrobial Activity of Spices' Extracts

Antimicrobial activity was determined by cup diffusion method on nutrient agar medium (Mohana *et al*;2008).The sterile medium was allowed to cool and plates were heavily with test bacterial cells. Three wells each of 5mm diameter were made in each plate. The desired concentration of each of the extracts in 50µl volume was introduced into wells in NA plates. All test plates were incubated at 37^0C for 24h Zones of inhibition were read using micrometer screw guage.

Determination of Minimum Inhibitory Concentration

The various solid extracts were reconstituted in ethanol and diluted with nutrient broth to yield concentrations of 5.00mg/mL,10.00mg/mL, 15.00mg/mL and 20.00mg/mL in different test tubes. The test tubes were each inoculated with 0.50mL(7.00log $_{10}$cfu/mL) of overnight broth culture of the test organisms using calibrated Pasteaur pipette and incubated at 37^0C for 24h. Control experiments were also set up but without the extracts. The least concentration of the extracts showing clear zone of inhibition was taken as the minimum inhibitory concentration(MIC).

Determination Minimum Bactericidal Concentration:

Tubes that did not show visible growth from the minimum inhibitory concentration experiments were sub-cultured unto nutrient agar (Oxoid) and incubated at 37^0C for 24h. The least concentration of the extracts that inhibited the growth of the test organisms were taken as the minimum bactericidal concentrations (MBC).

Table 5. Percentage (%) inhibition of bacteria by the extracts

Extracts	Test organisms(Isolates)						
	E. coli	***P. pneumoniae***	***B. cereus***	***K. aerogenes***	***S. aureus***	***S. typhi***	***Enterbactersp***
Nutmeg	36.70	30.40	32.40	33.50	31.60	29.30	30.80
Pepper	32.30	30.40	38.60	31.50	28.90	30.50	31.40
Ginger	30.30	33.40	32.60	32.40	31.80	32.70	33.60
Garlic	29.00	30.10	26.40	31.32	30.30	30.20	31.50

Determination of Percentage Inhibition of Test Organisms

Broth solutions of 20.00mg/ml of each extract in different test tubes were inoculated with 24h old cultures of the various test organisms as described by Akujobi et al; (2006).Tubes were thereafter incubated at37^0C for 24hs. Absorbance was read from a spectrophotometer for both experiment and control without the extracts. Percentage inhibition was calculated from the relationship

Percentage inhibition=$\frac{AC-AT \times 100}{AC}$

AC = absorbance of control . AT =absorbance of test sample (experiment).

RESULTS

A total of 7 bacterial species were isolated from all the meat samples. These include *Escherichia coli, Pseudomonas pneumoniae, Bacillus cereus* and *Klebsiella aerogenes.* Others are *Staphylococcus aureus, Salmonella typhi* and *Enterobacter* sp*. E. coli* and *Enterobacter* sp were isolated from all the meat samples,*B. cereus* was isolated from chicken and pork*, P. pneumoniae* occurred only in pork. *Klebsiella aerogenes* was isolated from beef and pork*, Salmonella typhi* and *Staphylococcus aureus* were obtained from beef, pork and goat meat (Table 1)

The effect of the spices' extracts on growth of the microorganisms measured as zones of inhibition is shown in Table 2. All spices showed inhibitions to the organisms to varying degrees*. Klebsiella aerogenes* showed the least sensitivity to all the extracts while *Salmonella typhi* showed the highest sensitivity as recorded with nutmeg*. E. coli* which was the most encountered was more sensitive to ginger with African Ethiopian pepper demonstrating the least sensitivity to the test organisms except with *S. typhi*.

The result of the minimum inhibitory concentrations (MIC) is shown in Table 3. The MIC was 15.00mg/mL for all extracts on the organisms except that of nutmeg on *P.pneumoniae* and *S. typhi*, African Ethiopian pepper on *Staphylococcus aureus, ginger* on *E.coli* and garlic on *Bacillus cereus* with MIC value of 10.00mg/mL each.

Minimum bactericidal concentrations (MBC) of the extracts on the test organisms are shown in table 4. The highest MBC value obtained was 20.00mg/mL for different extracts on various organisms while the lowest was 15.00mg/mL. The MBC for garlic was 20.00mg/mL for all the test organisms.

The percentage inhibition of the test organisms is shown in Table 5. The highest value of 38.60% was recorded with African Ethiopian pepper extract on *Bacillus cereus* while the lowest of 26.40% was recorded for garlic also on *Bacillus cereus*.

DISCUSSION

The presence of antimicrobial substances in spices and herbs has been reported (Irobi, 1992; Ibekwe et al; 2004 Nwafor and Ogiehor 2008). The present study has demonstrated the inhibitory effect of extracts of spices against *Escherichia coli, Pseudomonas pneumoniae, Bacillus cereus, Klebsiella aerogenes, Staphylococcus aureus, Staphylococcus typhi and Enterobaceter* sp isolated from meat. This is an indication that extracts contains substances that can inhibit the growth of the bacteria as shown by the zones of inhibition (Table 1) which differ for different bacterial species and extracts. This is in line with an earlier report by Zhang *et al*., (2005).They reported that the degree of antimicrobial activity of plant extracts depended on the type of bioactive ingredients in them. Some of these bioactive ingredients have been studied to include alkaloids, cyanogenic glycosides, saponins, tannins and oxalates (Akujobi et al., 2006; Celicel and Kavas, 2008; Adegoke *et al*., 2010;).The minimum inhibitory concentration is seen to be concentration dependent. Thus the higher concentrations showed higher inhibitions as shown by lower counts obtained (results not shown). Similar results have been obtained by Obi and Onuoha (2000). This effect could have resulted from injuries or distorted homeostasis or other negative effects of the extracts on the bacteria and agrees with the previous findings of Nwafor and Ogiehor (2008).

The extracts have shown the ability to inhibit the growth

of *Staphycoccus aureus, Pseudomonas pneumoniae , Klebsiella aerogenes and Escheichia coli*some which have been known to be multidrug resistant (Adegoke and Adebayo –tayo,2008). Multi drug resistance is thought to be due to the ability of microorganisms to either inactivate the active components of the drugs or the presence in them of the mechanisms for converting toxic substances to non or less toxic ones (Adegoke and Adebayo-Tayo 2009a 2009b).It therefore implies that these organisms may not have been able to inactivate or convert the active components of the extracts to non- toxic substances hence the growth inhibition recorded.

This study has shown that with proper application, extracts of these spices can be used for the preservation of meat at ambient temperature. This venture will be worthwhile particularly in Nigeria where cost of refrigeration is very high.

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