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Full Length Research Paper

Antimicrobial properties of three spices used in the preparation of *suya* condiment against organisms isolated from formulated samples and individual ingredients

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The effects of *Eugenia aromatica* (clove), *Allium sativum* (garlic) and *Piper guineense* (brown pepper), three spices commonly used in south-western Nigeria, added to growth media at different concentrations were studied with respect to their inhibitory activity against *Bacillus cereus, Bacillus coagulans, Bacillus enterobacter* sp.*, Aspergillus niger* and *Rhizopus stolonifer* isolated from already formulated and individual spices/ingredients used in the preparation of *suya* pepper. The sensitivity of isolated organisms revealed that clove is outstanding compared to the much worked on garlic and that gram positive bacteria showed higher sensitivity to spices than gram negative bacteria. *R. stolonifer* showed a higher sensitivity to brown pepper than it did to garlic. The growth of *A. niger* was not completely inhibited by brown pepper or a combination of both. The percentage composition of these three spices affected their inhibitory effects on microorganisms in *suya* condiment.

Key words: *Suya*, condiment, spices.

INTRODUCTION

Suya condiment is a mixture of spices and flavourings used for West African kebabs. *Suya* originated in Hausa land, Northern Nigeria, but is now popular in many African countries. Billing and Sherman (1998) have enumerated the frequency of use of 43 spices in meat based cuisines of the 36 countries for which traditional cookbooks were located. Early studies demonstrated that spices had high population of bacteria (Yasair and Williams, 1942) that remained viable even at the time of marketing (Fabian et al., 1939). This situation seems to have prevailed to the present (Powers et al., 1975). Spores of both *Clostridium perfringens and Bacillus cereus* have been found to be present in spices and herbs (Kneifel and Berger, 1994; Perfumi, 1986).

Not all the methods of decontamination can be used on spices; some are ineffective, others are effective but destroy the spices. For example, ultra violet light does not penetrate the surface of spices and if sterilized by dry heat, spices become charred (Farag and Abo-Zeid, 1997). If sterilized by steam, they lose volatile components. Steam treatment of pepper for 5 min at 5 - 15 pounds steam pressure decreases the potency of its flavour by 10% (Yasair and Williams, 1942). The use of ethylene oxide in a sealed warehouse is now banned in EU countries due to the toxicity and extreme inflammability of ethylene oxide (Uiji, 1992).

Studies in the past decade confirm that the growth of both gram-positive and -negative foodborne bacteria, yeast and mold can be inhibited by spices. Nwaiwu and Imo (1999) have screened 3 Nigerian spices; "Ehuru" (African nutmeg- *Monodora myristica); "*Uziza" (*Piper guineense)* and "Uda" (*Xylopia aethiopica)* for the fungitixicity of their essential oils against mycelial growth of 3 food-borne fungi; *Aspergillus fumigatus, Aspergillus nidulans* and *Mucor hiemalis*. The essential oils from all the spices were fungi-toxic at varying degree to all the organisms. Inhibition of *Salmonella typhimurium* and *Escherichia coli* by reconstituted onion and garlic powder was reported by Johnson and Vaughn (1967). According to Shelef (1983), garlic inhibited *S. typhimurium, E. coli,*

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Staphylococcus aureus, B. cereus, Bacillus subtilis, mycotoxigenic *Aspergillus* and *Candida albicans.* The extract of *Eugenia aromatica* (clove) incorporated in potato dextrose agar (PDA) provided inhibitory activity against *Alternaria* sp., *Fusarium sp., Botrytis* sp. and *Septoria* sp. at varying concentrations (Soatthiamroong et al., 2004).

Although, the antimicrobial activity of some spices and herbs is documented (Giese, 1994), the normal amounts added to foods for flavour are usually not sufficient to completely inhibit microbial growth. Antimicrobial activity varies widely, depending on the type of spices or herb, test medium and microorganisms. For this reason, addition of spices should not be considered as a primary preservative method (Giese, 1994).

It is now recognized that spices and herbs may fulfill more than one function in foods to which they are added. In addition to imparting flavour, certain spices prolong the storage life of foods by a bacteriostatic or bactericidal activity. Being natural foodstuffs, they appeal to consumers who tend to question the safety of synthetic food additives (Shelef et al., 1980).

The aim of this investigation was to establish the antimicrobial effect of three spices against organisms isolated from the spices and determine the quantity of the spices needed to inhibit the growth of microorganisms.

MATERIALS AND METHODS

Samples and organisms

Already prepared *suya* condiment and the various ingredients used for its preparation were purchased at the Sabo area of Ibadan, Nigeria. They were free of obvious diseases. These were identified at the herbarium of the Botany and Microbiology Department, University of Ibadan, Ibadan, Nigeria.

Isolation

Individual spices purchased along side the formulated sample were stored in sterile airtight containers. The spices were subsequently ground in a grinder (Corona) to fine powder and the colony forming unit (CFU/g) of each spice and other ingredients were determined with a plate count agar (PCA) and potato dextrose agar (PDA) for bacterial and fungi, respectively, using the pour plate method after serial dilution ($10^{-1} – 10^{-5}$) and incubated at ambient temperature (29 ± 2°C) for 24 – 36 h and 48 – 72 h for bacterial and fungi, respectively. The grinder was surface sterilized after each use with 75% ethanol.

Drying of garlic (*Allium sativum*)

Sliced fresh cloves of garlic were dried at 45°C for 3 - 4 days (Staba et al., 2001).

Characterization and identification

Pure cultures of bacteria isolated were characterized and identified on the basis of their cultural, morphological and biochemical properties and by reference to Bergey's Manual of Determinative Bacteriology, (Holt et al., 1994). The fungi isolated were characterized based on their macroscopic appearance on the culture medium, microscopic morphology and type of asexual spores produced and identified by reference to the Compendium of soil fungi (Domsch and Gams, 1980).

Screening of spices for inhibitory activity against isolated organisms

Finely ground powder of *E. aromatica* (clove), *A. sativum* (garlic) and *P. guineese* (brown pepper) were added at 3 different concentrations (0.5, 1.5 and 3.0%) to each culture medium before autoclaving at 121°C for 15 min. The pour plate method was used after the introduction of 0.1 m aliquots of serial dilution of 18 – 24 h bacteria and 48 – 72 h fungi cultures in a nutrient agar (NA) and potato dextrose agar (PDA) containing the various spice concentrations (Shelef et al., 1980). Plates without spices served as standard while plates containing spices but no organisms served as control. All plates were incubated at ambient temperature (28 – 30°C) and counted after 24 h and 48 h.

Screening of spice combination for inhibitory activity against isolated organisms

Nutrient agar and potato dextrose agar plates with spice combination of clove, garlic and brown pepper in the ratio 1:1:1 and that of garlic and brown pepper in the ratio 1:1 were prepared. These spice combinations were investigated as earlier described for individual spices.

RESULTS AND DISCUSSION

Results of the colony counts from the individual spices and other ingredients of *suya* condiment are presented in Figure 1. No growth was observed in the growth medium containing clove, garlic and brown pepper. Giese (1994) reported that few microorganisms were present in spices with higher antimicrobial activity such as sage, cloves and oregano. Zaika (1988) on his part classified clove, cinnamon and mustard as strong antimicrobial spices while sage and oregano were among those with moderate antimicrobial effects. Red pepper and ginger were classified as having weak antimicrobial effects. The absence of growth in the media containing these spices may not be unconnected with the inhibitory effects carried over with the inoculum. It was generally noticed that in plates where growth occurred, low colony counts were observed on the low dilution plates while higher counts were obtained on the higher dilution plates, a direct opposite of the expectation from serial dilution.

The bacteria isolated from the samples were *B. cereus, Bacillus coagulans, Bacillus megaterium* and *Enterobacter* sp. The fungi isolated were *A. niger* and *R. stolonifer.* From Tables 1 and 2, it can be seen that clove inhibited the growth of all the organisms isolated from the individual ingredients and the formulated sample at all concentrations used unlike garlic and brown pepper that had their inhibitory effects at higher concentrations. Garlic inhibited *A. niger* above 0.5% and *R. stolonifer* above 1.5%. Brown pepper only inhibited *R. stolonifer* at concentrations above 0.5%.

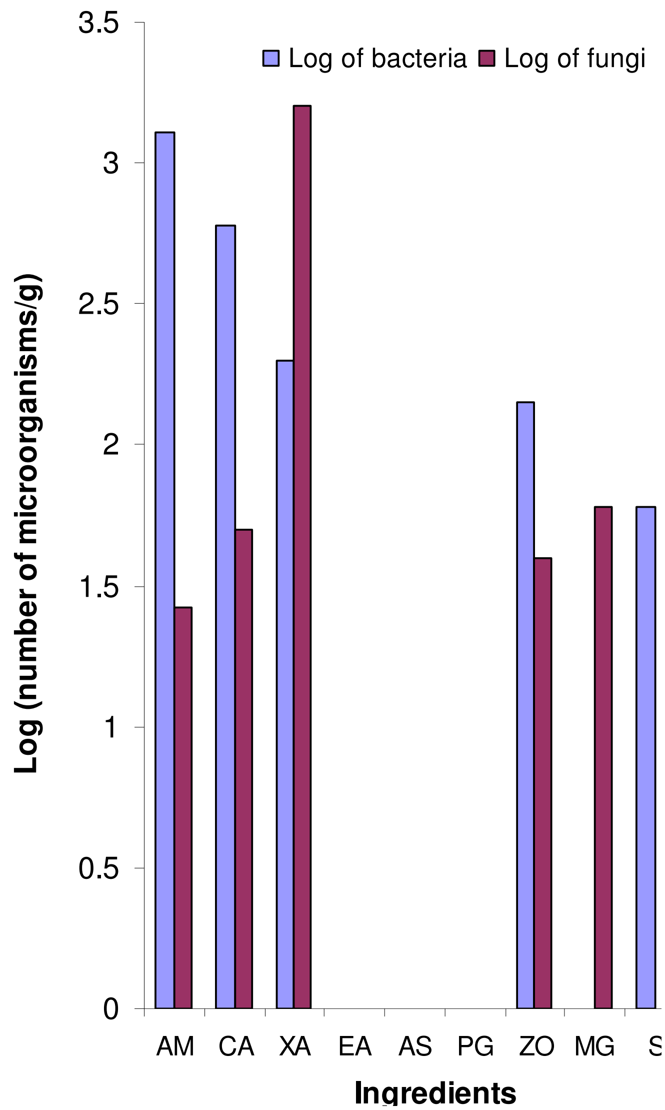

Figure 1. Colony count of isolates from individual ingredients normally used in suya condiment. AM - *Aframomium melegueta*; CA - *C. annuum*; XA - *X. aethiopica*; EA - *E. aromatica*; AS – *A. sativum*; PG – *P. guineese*; ZO – *Z. officinalis*; MG - monosodium glutamate; S - salt.

Table 1. Effect of ground clove, garlic and brown pepper on bacterial growth on nutrient agar.

Bacteria	No spice	Spice concentrations (%)								
		Clove			Garlic			Brown pepper		
		0.5	1.5	3.0	0.5	1.5	3.0	0.5	1.5	3.0
B. cereus	xxx	-	-	-	xxx	x	-	xxx	-	-
B. cereus	xxx	-	-	-	xx	x	-	xxx	x	-
B. megaterium	xxx	-	-	-	xx	x	-	x	-	-
B. coagulans	xxx	-	-	-	xxx	xx	-	xxx	-	-
B. coagulans	xxx	-	-	-	xxx	xx	-	xxx	-	-
Enterobacter sp.	xxx	-	-	-	xxx	xx	x	xxx	xx	-
Enterobacter sp.	xxx	-	-	-	xxx	xx	x	xxx	xx	-

xxx: Abundant growth; xx: growth (numerous separate colonies); x: limited growth; - : no growth.

Table 2. Effect of ground clove, garlic and brown pepper on fungal growth on potato dextrose agar.

Fungi	No spice	Spice concentrations (%)								
		Clove			Garlic	Brown pepper				
		0.5	1.5	3.0	0.5	1.5	3.0	0.5	1.5	3.0
A. niger	xxx	-	-	-	xx	-	-	xxx	xx	xx
R. stolonifer	xxx	-	-	-	xxx	xx	-	x	-	-

xxx: Abundant growth; xx: growth (numerous separate colonies); x: limited growth; - : no growth.

Table 3. Percentage composition of *suya* condiment from South-western Nigeria.

Ingredients	Market range (g)	Average	Composition (%)
E. aromatica	7.0 – 7.9	7.48	2.25
P. guineense	5.6 – 6.6	6.10	1.84
A. melegueta	17.5 – 19.4	18.45	5.58
X. aethiopica	7.7 – 8.4	8.05	2.43
A. sativum	24.4 – 26.2	25.30	7.65
Z. officinalis	38.7 – 52.8	45.75	13.83
C. annuum	150.2 – 189.4	169.80	51.32
Monosodium glutamate	50.0	50.00	15.11
Salt	to taste		

Table 4. Effect of combined spices on bacterial growth in nutrient agar.

Bacteria	No spice	Spice concentrations (%)					
		Brown pepper, clove and garlic			Brown pepper and garlic		
		0.5	1.5	3.0	0.5	1.5	3.0
B. cereus	xxx	xx	-	-	xx	x	-
B. cereus	xxx	xx	-	-	xx	x	-
B. megaterium	xxx	-	-	-	xx	x	-
B. coagulans	xxx	-	-	-	xx	-	-
B. coagulans	xxx	-	-	-	xx	-	-
Enterobacter sp.	xxx	-	-	-	xx	xx	-
Enterobacter sp.	xxx	-	-	-	xx	xx	-

xxx: Abundant growth; xx: growth (numerous separate colonies); x: limited growth; - : no growth.

The percentage composition of the spices and other ingredients used for the preparation of *suya* condiment at the Sabo area of Ibadan is presented in Table 3. The percentage composition of *E. aromatica* (clove), *A. sativum* (garlic) and *P. guineese* (brown pepper) is low when compared to those of *Aframomum meleguetas, Zingiber officinalis* and *Capsicum annuum. C. annuum* with the highest percentage of 51.32 exerts mild inhibitory effects on microorganisms in food. *Z. officinalis* which is next (113.3%) is bacteriostatic due to the terpen oil which binds to the bacterial protein (Uraih, 1997). So we see that the quantity of the more potent spices in the formulation is insufficient to eliminate or even inhibit the growth of microorganisms. The synergistic effect of a combination of the 3 spices in the growth media gave almost the same result as clove alone but a combination of garlic and brown pepper appeared not to enhance the antimicrobial effect on the isolated organisms. This is shown in Tables 4 and 5.

The sensitivity of the bacterial isolates to the spices revealed that the gram-positive bacteria showed higher sensitivity to the spices than the gram-negative bacteria (Shelef et al., 1980). It is not known exactly why gram-negative bacteria should be less susceptible, but it may be related to its outer membrane which endows the bacterial surface with strong hydrophilicity and acts as a strong permeability barrier (Nikaido and Vaare, 1985).

The low antimicrobial activity of garlic powder relative to clove powder was somewhat surprising since report has it that its antimicrobial activity exceeds that of most

Table 5. Effect of combined spices on fungal growth in potato dextrose agar.

Bacteria	No spice	Spice concentrations (%)					
		Brown pepper, clove and garlic			Brown pepper and garlic		
		0.5	1.5	3.0	0.5	1.5	3.0
A. niger	xxx	-	-	-	xx	x	x
R. stolonifer	xxx	-	-	-	x	-	-

xxx: Abundant growth; xx: growth (numerous separate colonies); x: limited growth; - : no growth.

broad spectrum antibiotics (Rees et al., 1993). They further stated that garlic inhibits different micro-organisms depending on its concentration in the growth environment. Hafnawy et al. (1993) has also observed the enhanced effect of garlic powder compared with other spices against *Listeria monocytogenes.* One cannot rule out the possible effect of heat during the drying of garlic clove (Staba et al., 2001).

The inhibitory effect of *E. aromatica* (clove) used as food additive should be of importance in medicine. Further work to reveal the active ingredient in clove will be useful in using it in pharmaceutical and food industries to increase shelf-life and decrease the possibility of food poisoning and spoilage in processed foods and drugs.

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