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Antibacterial Properties of Extracts of Some Nigerian Spices.

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The antibacterial properties of three Nigerian spices - *Zingiber officinale* (ginger), *Allium sativum* (garlic) and *Piper guineense* (African cola) - were tested against seven bacterial species. Extraction was done using ethanol and ethyl acetate for each of the spices. Extracts from *Z. officinale* and *P. guineense* showed less antimicrobial effects when compared with that of *A. sativum*. The extracts from *A. sativum* and *Z. officinale* were active against all microorganisms with zone of inhibition ranging from 0.2mm to 18.0mm. In general, the ethyl acetate extracts showed more activity then the ethanol extracts. Phytochemical screenings revealed the presence of only saponins in *Z. officinale*; saponins, tannins, phenolics and reducing compounds and alkaloids in *Allium sativum* and tannins, phenolics, reducing compounds and alkaloids in *P. guineeense*. The significance of the results obtained in this study are discussed.

Keywords: Antibacterial properties, Nigerian Spices.

Infectious diseases are a leading cause of morbidity and mortality in Africa (Sofoworola, 1982). The development of broad-spectrum antibiotics for the treatment of infections is a major breakthrough in medicine (Brooks, 2002). Despite the numerous advances in medicine, the prevalence of infectious diseases continues to rise due to the emergence of antibiotic-resistant pathogens, which are attributed to the widespread use of these antibiotics (WHO, 1999).

Spices are aromatic vegetable plants of tropical origin. The use of plants and herbs for in traditional medicine for the treatment of infections has been an age long practice (Kochhar, 1986; Sofoworola, 1982). In Nigeria, many plants - mostly in the form of herbs - are used for diverse medical purposes, which often reflect the diverse ethnic and cultural differences (Onyeagbe *et al.,* 2004). However, the advent of modern medicine and development of antibiotics for treatment of infections have suppressed the use of these plants.

Due to the increasing resistance of pathogens to modern antibiotics, scientists have been creating awareness to resuscitate the use of those plants, with confirmed antimicrobial properties, for treatment of infections while there are intense efforts to screen new plants for their antimicrobial properties - which could be useful in the future.

Presently, there is increasing evidence for the antimicrobial properties of various plants – including *A. sativum, Z. officinale* and *P. guineense*. Although studies on the medicinal uses and phytochemistry of *P. guineense* are very sparse, the phytochemistry and antimicrobial properties of *A. sativum* and *Z. officinalle* have been reported (Masido *et al.,* 1989; Bose *et al.,* 1990; Duke and Reckstrom - Syernberg, 1991; Farbman *et al.,* 1993; Philips *et al.,* 1993; Bordia *et al.,* 1996; Rabinkou *et al.,* 1998 and Ankn and Mirelman, 1999). According to Robbers, (1996), plant materials are present in, or have provided models for 50% of western drugs and are now gradually being integrated into orthodox medicine – especially in Africa.

In the present study, the antibacterial properties of ethanol and ethyl acetate extracts of three Nigerian spices - *Zingiber officinale, Allium sativum* and *Piper guineenese* were investigated.

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MATERIALS AND METHODS

Plant Materials

The plant of *Zingiber officinale, Allium sativum* and *Piper guineenese* were collected in Ado – Ekiti, Ekiti State, Nigeria. The identification of plants was done at the herbarium unit, Department of Plant Science, University of Ado- Ekiti, Ekiti State, Nigeria. Voucher specimens were deposited at this unit.

Preparation of Extracts

Powdered air-dried plant material was extracted with ethanol and ethyl acetate. The crude methanol extracts was prepared by maceration of material (100g) with 70% ethanol or ethyl acetate (300ml) (Alanis *et al.,* 2005).

Clinical Strains

The test organisms were all clinical strains of *Escherichia coli, Staphylococcus aureus, Klebsiella pneumoniae, Pseudomonas aeruginosa, Salmonella typhi, Proteus vulgaris* and *Bacillus cereus.* They were all obtained from the stock culture collection of the Department of Microbiology, University of Ado-Ekiti, Ekiti-State, Nigeria.

Antibacterial Assay

Antibacterial activity was measured using agar dilution technique as described by Alanis *et al,* (2005). The ethanol and ethyl acetate extracts were reconstituted in dimethyl sulfoxide (DMSO, Merck) and serially diluted in molten Mueller Hinton agar (MHA, Sigma) in petridishes (100mm x 15mm) to obtain final concentrations: 125, 62.5, 31.25 and 15.625 µg/mL. The solvent did not exceed 1% concentration and did not affect the growth of the test organisms. They were grown in Mueller Hinton broth (MHB, Sigma) for 4h at 37^{o}C. Bacterial suspensions with 0.5 McFarland standard turbidity, which is equivalent to 10^8 cfu/mL, were prepared by dilution with Mueller Hinton broth. The diluted inoculum was added to a Steer's replicator calibrated and incubated for 24h at 37^{o}C. After incubation, all dishes were observed for zones of inhibition and the diameters of these zones were measured in millimeters. The minimum inhibiting concentration (MIC) was determined as the lowest concentration that completely inhibited macroscopic growth of the organisms.

Phytochemical Screening

The three Nigerian spices were screened for some phytochemicals such as saponin, tannin and phenolics, reducing compounds and alkaloids. The method described by Sofowora (1992) was adopted for the screening.

RESULTS

The results showed a wide variation in the properties of the extracts from the spices by demonstrating a broad spectrum against both Gram positive and Gram-negative bacteria and *Candida albicans*.

All the bacteria were more sensitive to ethyl acetate extracts than ethanol extracts at varying concentrations (12.5 µg/ml-15.625µg/ml). *Salmonella typhi*, *Staphylococcus aureus, Bacillus cereus* and *Proteus vulgaris* were more sensitive to ethanol extracts (12.5µg/ml-15.625 µg/ml), while *Candida albicans*, *Staphylococcus aureus*, *Proteus vulgaris*, *Pseudomonas aeruginosa* and *Salmonella typhi, Proteus*

Table 1. Antimicrobial activity of ethanol extracts of *A. sativum, Z. officinale* and *P. guineense.*

Plants/ Test organisms	*Z. officinale*				*A. sativum*				*P. guineense*			
Conc. (µg/ml)	12.5	62.5	31.25	15.625	12.5	62.5	31.25	15.625	12.5	62.5	31.25	15.625
E. coli	7.0	6.0	5.0	4.0	8.0	6.0	4.0	2.0	7.0	6.0	5.0	3.0
S. aureus	10.0	8.0	7.0	4.0	11.0	10.0	9.0	7.0	9.0	8.0	6.0	5.0
K. pneumonia	6.0	5.0	4.0	3.0	8.0	6.0	4.0	2.0	5.0	3.0	1.0	0
P. aeruginosa	7.0	6.0	5.0	3.0	11.0	9.0	7.0	5.0	10.0	9.0	8.0	7.0
S. typhi	12.0	10.0	9.0	6.0	9.0	8.0	7.0	6.0	7.0	5.0	0	0
P. vulgaris	5.0	4.5	4.0	0	10.0	8.0	6.0	5.0	13.0	11.0	10.0	9.0
B. cereus	10.0	9.0	5.0	4.0	12.0	10.0	8.0	6.0	7.0	5.0	3.0	1.0

Zone of inhibition in (mm).

Table 2. Antimicrobial activity of ethyl acetate extracts of *A. sativum, Z. officinale* and *P. guineense.*

Plants/ Test organisms	*Z. officinale*				*A. sativum*				*P. guineense*			
Conc. (µg/ml)	12.5	62.5	31.25	15.625	12.5	62.5	31.25	15.625	12.5	62.5	31.25	15.625
E. coli	9.0	8.0	7.0	6.0	12.0	10.0	8.0	6.0	6.0	5.5	4.0	3.0
S. aureus	12.5	12.0	11.0	10.0	12.0	8.0	6.0	5.0	7.0	6.0	4.0	3.5
K. pneumonia	8.0	7.0	6.0	5.0	14.0	12.0	11.0	9.0	5.0	4.0	3.5	3.0
P. aeruginosa	7.0	6.0	5.0	4.0	17.0	15.0	14.0	10.0	12.0	10.0	8.0	6.0
S. typhi	6.0	5.0	0	0	12.0	10.0	9.0	8.0	12.0	9.0	7.0	4.5
P. vulgaris	7.0	6.0	4.0	0	18.0	16.0	14.0	12.0	9.0	7.0	6.5	5.0
B. cereus	9.0	8.0	7.5	7.0	14.0	12.0	11.0	9.0	9.0	7.0	6.0	5.0

Zone of inhibition in (mm).

vulgaris were more susceptible to ethyl acetate extracts. (Tables 1 & 2).

However the result presented in Table 2, showed that the ethyl acetate extract of Z *officinale*, *A. sativum* and *P. guineense* posses high antimicrobial activity against all test organisms.

The plants differ significantly in their activity against the tested organisms and the best antimicrobial activity was observed at 12.5 µg/ml. From the three tested spices, both extracts from *A. sativum* and *Z .officinale* were active against all microorganisms with zones of inhibition ranging from 2.0mm to 18.0mm.

Extracts from *A. Sativum* and *Z. officinale* exhibited a strong activity against *S. typhi*, *B. cereus* and *P. vulgaris* with zones of inhibition ranging from 12.0mm to 18.0mm. The highest antimicrobial activity was observed with ethyl acetate extract of *Allium sativum.* It inhibited the growth of all the test organisms.

The results of phytochemical screening of these plants were presented in Table 3. *Z. officinale* was found to contain saponins, tannins, phenolics and reducing compounds but not alkaloids. The screening of *P. guineense* revealed the presence of tannins, phenolics and reducing compounds and alkaloids.

Table 3. Phytochemistry of *A. sativum, Z. officinale* and *P. guineense.*

Components	*Z. officinale*	*A. sativum*	*P. guineense*
Saponins	+	+	-
Tannins and Phenolics	-	+	+
Reducing Compounds	-	+	+
Alkaloids	-	-	+

DISCUSSION

For decades, *A. sativum, Z. officinale* and *P. guineense* have been used for medicinal purposes in African tradition medicine (Iwu and Kokwaro, 1996). This study has confirmed that these spices possess antimicrobial properties. Some studies have also reported the antimicrobial effect of these plants and this suggests that they could be very good options as alternative remedy for treatment of infectious and other medicinal uses (Sofoworola, 1982; Boakye-Yiadom *et al.,* 1977; Iwe, 1993; Bacquer, 1995).

In this study, *A. sativum* was most active against the test organisms, based on the zones of inhibition ranging from 5.0mm - 18.0mm, with the highest activity obtained at 125µg/mL. This was immediately followed by extracts of *Z. officinale* and *P. guineense* (Tables 1 and 2). Alanis *et al,* (2005), screened several plants and *A. sativum* was found to possess a high activity against enteric pathogens. In this study, the phytochemical components observed, which include tannins, phenolics, reducing agents and alkaloids, could be responsible for the high antibacterial activity of these plants. This is in accord with the findings of Dagne, (1996).

It was observed that the ethyl acetate extracts of the three spices (Table 2) showed a higher activity than their corresponding ethanol extracts (Table 1). This suggests that ethyl acetate could be a better solvent for extraction. The best concentration for antimicrobial activity for both solvents was 125mg/mL as all the extract showed activity against all the organisms at this concentration.

This study examined the different susceptibilities of the bacteria. *E. coli, S. aureus* and *P. vulgaris* were most sensitive to ethanol extracts of *Z. officinale, A. sativum* and *P. guineense* respectively (Table 1). Similarly, *S. aureus, P. vulgaris* and *P. aeruginosa* were most sensitive to ethyl acetate extracts of *Z. officinale, A. sativum* and *P. guineense* (Table 2). The five enteric bacteria showed high sensitivity to the extracts. This justifies their use, particularly Z. *officinale* and *A. sativum* as remedy for gastrointestinal disorders. Also, the activity of the plants against *S. aureus* indicates that they could be used for treatment of skin infections.

In conclusion, the antimicrobial activity of these spices observed in this study justifies their use in African traditional medicine and this should facilitate routine screening of plants that could be used for alternative remedy for serious health problems.

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