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 than 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. guineense. 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. officinale 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 guineense were investigated.

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MATERIALS AND METHODS

Plant Materials
The plant of *Zingiber officinale*, *Allium sativum* and *Piper guineense* 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°C. Bacterial suspensions with 0.5 McFarland standard turbidity, which is equivalent to 10⁸ 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°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*.

<table>
<thead>
<tr>
<th>Plants/Test organisms</th>
<th><em>Z. officinale</em></th>
<th><em>A. sativum</em></th>
<th><em>P. guineense</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Conc. (µg/ml)</td>
<td>12.5</td>
<td>62.5</td>
<td>31.25</td>
</tr>
<tr>
<td><em>E. coli</em></td>
<td>7.0</td>
<td>6.0</td>
<td>5.0</td>
</tr>
<tr>
<td><em>S. aureus</em></td>
<td>10.0</td>
<td>8.0</td>
<td>7.0</td>
</tr>
<tr>
<td><em>K. pneumonia</em></td>
<td>6.0</td>
<td>5.0</td>
<td>4.0</td>
</tr>
<tr>
<td><em>P. aeruginosa</em></td>
<td>7.0</td>
<td>6.0</td>
<td>5.0</td>
</tr>
<tr>
<td><em>S. typhi</em></td>
<td>12.0</td>
<td>10.0</td>
<td>9.0</td>
</tr>
<tr>
<td><em>P. vulgaris</em></td>
<td>5.0</td>
<td>4.5</td>
<td>4.0</td>
</tr>
<tr>
<td><em>B. cereus</em></td>
<td>10.0</td>
<td>9.0</td>
<td>5.0</td>
</tr>
</tbody>
</table>

Zone of inhibition in (mm).
Table 2. Antimicrobial activity of ethyl acetate extracts of A. sativum, Z. officinale and P. guineense.

<table>
<thead>
<tr>
<th>Plants/ Test organisms</th>
<th>Z. officinale</th>
<th>A. sativum</th>
<th>P. guineense</th>
</tr>
</thead>
<tbody>
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</tr>
<tr>
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</tr>
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<td>6.0 5.0</td>
</tr>
<tr>
<td>P. aeruginosa</td>
<td>7.0</td>
<td>6.0</td>
<td>5.0 4.0</td>
</tr>
<tr>
<td>S. typhi</td>
<td>6.0</td>
<td>5.0</td>
<td>0 0</td>
</tr>
<tr>
<td>P. vulgaris</td>
<td>7.0</td>
<td>6.0</td>
<td>4.0 0</td>
</tr>
<tr>
<td>B. cereus</td>
<td>9.0</td>
<td>8.0</td>
<td>7.5 7.0</td>
</tr>
</tbody>
</table>

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.

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

<table>
<thead>
<tr>
<th>Components</th>
<th>Z. officinale</th>
<th>A. sativum</th>
<th>P. guineense</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saponins</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Tannins and Phenolics</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Reducing Compounds</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Alkaloids</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
</tbody>
</table>

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.

REFERENCES
