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# Antibacterial Activities of Some Commercially Available Herbal Remedies in Owerri Imo State

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**Abstract**

The aim of the present study was to investigate the antibacterial activities of 10 different herbal medicinal products sourced from the traditional medicine sales outlets and at the traditional medical trade fare in Imo State of Nigeria. The streaking and the agar-well diffusion methods were adopted for the inoculation of test organisms and sensitivity testing respectively. The antibacterial activities of the products varied considerably. From the screening experiments, product H showed the best antibacterial activity while production B had the least antibacterial activity by inhibiting only Escherichia coli with zone diameter of 16.75+0.01mm and MIC/MBC index of 0.50. The highest zone of inhibition was obtained with product D against Staphylococcus aeurus (26.45+0.06mm) and the MIC value of 50.0mg/ml. Pseudomonas aeruginosa showed a high level of resistance to the products and was slightly inhibited by only product G with zone diameter of 3.78+0.14mm. There is need to standardize, monitor and regulate the product of herbal remedies available in the Nigerian markets.

**Key words:** Antibacterial activity, commercially available, herbal remedies

## 1. Introduction

Nature is one way or the other has provided answers for every illness and disease conditions known to man. "Plants" have been outstanding in this regard (R'ios and Recio, 2005). The use of medicinal plants for the treatment of illness is in practice in many parts of the world. Every country has its own indigenous medicinal system (Awosika, 1995). Herbal therapy is becoming increasingly popular among patients and physicians. Many herbal preparations are marketed to the public for various ailments (Krim *et al*, 2002). Currently especially in the United State, herbal products as not regulated as they are considered dietary supplements (Routh and Bhowmik, 1999). Therefore, there is no standardization of active ingredients, purity or concentration. There is also no regulations governing which herbs can be marketed for various ailments (Routh and Bhowmik, 1999). This has made learning about and using these treatments challenging. Information compiled in a practical fashion may enable more patients to benefit from these treatments currently used worldwide (Routh and Bhowmik, 1999).

The indiscriminate use of antibiotics has resulted in many bacterial pathogens rapidly becoming resistant to a number of the originally discovered antimicrobial drugs (Barbour *et al*, 2004). The antimicrobial compounds found in plants may prevent bacterial infections by different mechanisms than the commercial antibiotics and therefore may have clinical value in treating resistant microorganism strains (Eloff, 1999). There is, thus, a continuous search for new antibiotics and medicinal plants may offer a new source of antibacterial agents.

Combination of two or more antibiotics with different mechanisms of action are sometimes tested in an attempt to improve efficacy through synergy and prevent the development of antibiotic resistance (Beringer, 1999). Many potential antimicrobial compounds are not singularly effective, and in combination with another compound, could possibly improve antimicrobial efficacy through synergism.

Moreover, the increase cost of new and more effective antimicrobial remedies (Salie, *et al* 1996) together with their side effects and lack of health care facilities in some rural areas (Griggs *et al*, 2001), makes the search for safer, more effective and affordable alternative remedies imperative.

The majority of medicinal plant consumers purchase herbal material from markets. Stafford *et al* (2004), state that approximately 450 plant species are sold in large volumes from herbal trade markets. These markets generally have poor physical conditions and infrastructures with a lack of storage facilities resulting in spoilage of plant material (Stafford *et al*, 2004, Mander, 1997). These poor conditions lead to wastage and a decrease in the quality of plant materials. Fennell *et al* (2004), state that Africa is fairly behind with regard to the control and development of the medicinal plant industry and researchers are, thus, focusing on several aspects needed for the development of the medicinal plant trade in Africa.

## 2.0 Materials and methods

**2.1 Collection of Samples:**

The traditionally (locally) made concoctions were collected from the herbalist at the tradomedical trade fare of the different zones in Imo State. The concoction collected includes those for the treatment of typhoid fever (product A and B-*Eucalyptus offensinalis* and *Azadirichta indica*) Diarrhoea (product C and D-*Psidum govaja* and *Anacardium occidentale*) Bronchitis (product E and F-*Cocos nucifera* and *Mangifera indica*) urinary tract infection (product G and H-*Ficus asperifolia* and *Alluim sativum*) and venereal diseases (product I and J-*Terminalis catappa* and *Musa paradisiaca*).

**2.2 Concentration of the herbal remedies and storage**

About 200ml of the herbal remedies of various plants were evaporated to dryness in a steady air current for 24hours. The fine residues were exposed to U.V. rays for 18 hours after which it was checked for sterility by streaking on nutrient agar plate. The residues were stored in different clean sterile labeled containers until they were needed.

**2.3 Collection, purification, confirmation and storage of the test organisms**

Clinical bacterial isolates were obtained from Federal Medical Centre Owerri microbiology laboratory. The organisms include *staphylococcus aureus, Escherichia coli, Streptococcus pneumoniae, Proteus mirabilis and Pseudomones aeruginosa.* These organisms were purified by re-isolating in Hinton agar and was confirmed after characterization by standard bacteriological methods (Cheesbrough, 2004). The stock cultures were stored at $4^oC$ in nutrient agar slants.

**2.4 Standardization of inoculums:**

The Mcfarland nepholometer standard was prepared according to the National committee for clinical laboratory standards (NCCLS, 1998). The final inoculum was adjusted to $1.0X10^6$cfu/ml for gram positive bacteria and $5X10^5$cfu/ml for gram negative bacteria.

**2.5 Sensitivity screening of test samples by agar well diffusion method at 200mg/ml:**

This was done following the method describes by Okoli *et al* (1988). The inoculum of each of the test organisms was seeded onto sterile Muller Hinton Agar plates. Subsequently, 100µl of 200mg/ml concentration of the various concoction extracts was separately introduced in dublicate well of the agar culture. The plates were allowed to stand for 1 hour to allow diffusion of the extracts to take place and then incubated for $37^oC$ for 24 hours. The zones of inhibition were recorded to the nearest millimeter (mm).

**2.6 Determination of minimum inhibitory concentration and minimum bactericidal concentration of the test samples:**

The MIC was determined by Macro-Broth Dilution techniques as specified by NCCLS (1998). A two fold serial dilution of the reconstituted extract was prepared in a Muller Hinton Broth. Each dilution was seeded with 100 µl of the standardized suspension of the test organisms (i.e $1.0X10^6$cfu/ml for Gm +ve and $5.0X10^5$ cfu/ml for Gm-ve bacteria). All the cultures were incubated for 24 hours at $37^oC$. MIC was determined as lowest concentration of the test samples that showed visible growth. For MBC, nine 0.1ml volume of Broth from each macro-Broth MIC testing showing no bacterial growth was taken and incubated in a sterile Muller Hinton agar at $37^oC$ for 24 hours. The MBC was determined as the least concentration showing no growth on subculture.

**2.7 Statistical analysis**

Paried sample T-test was conducted to analyze the diameter of the zone of inhibition. Values are reported as means of duplicate determination $\pm$ standard deviation.

**3. Results**

There was a significant difference ($P<0.05$) between the mean zone inhibition of the herbal remedies. Product H had antibacterial activities against the majority of the test organisms with mean zone of inhibition ranging from $12.16\pm0.02$mm to $25.57\pm0.13$mm. However the highest zone of inhibition of $26.45\pm0.06$mm was produced against *staphylococcus aureus* by product D. Only product G had activity against *Pseudomonas aeruginosa* with zone diameter of $3.78\pm0.14$mm. This is shown in Table 1. Product B had the least antibacterial activity. It only inhibited *E.coli* with zone diameter of $16.75\pm0.01$. The minimum inhibitory concentration (MIC) and minimum bacterdical concentration (MBC) of the extracts of the herbal remedies are shown in Table 2. The activity index measured as ratio of the MBC to MIC showed that product H had an index of 1.00 indicating that it had a bacteridical effect against *Escherichia coli*. The rest of the products had a bacteriostatic effect with activity index of less than 1.00. The MIC values of these extracts ranged from 50-100. The extracts of product E, G and H had bactericidal effect against *staphylococcus aureus* with MBC value ranging from 50mg/ml to 100mg/ml. Product

 

B exhibited no effect while the rest were bacteriostatic against *staphylococcus aureus* (index of 0.50).

Only product H showed a bactericidal effect against *Streptococcus pneumoniae* with an MBC value of 100mg/ml. Products G, H and I exhibited a bacteriostatic effect against *Proteus, mirabilis* with MIC value of 50-100mg/ml while product F had a bactericidal effect against *Proteus mirabilis* with an MBC value of 25mg/ml. *Pseudomonas aeruginosa* showed a high level of resistance to all the products.

## 4. DISCUSSION

The efficacy pattern of the herbal remedies used in the treatment of typhoid fever, diarrhea, bronchitis, urinary tract infection and venereal diseases, revealed varying level of antibacterial activities of each batch of traditional herbal remedy on the test hospital isolates.

Herbal drug preparation methods by traditional healers are mainly by maceration, infusion and decoction. "Drugs" prepared in this way most likely extract polysaccharides that could be responsible for mucosal layer protection by binding to the toxin when administers orally. During storage, preparations may undergo fermentation that enables enzymes activation or formation of decomposition products that in turn may kill the organism or neutralize the toxins when the drug is taken (Manegesi *et al*, 2008).

Varying levels of sensitivity ranging from resistance to slight, moderate and heavy inhibition were observed. This difference of activity appears to be directly related to the quantitative and/or qualitative diversity of the compounds that are being accumulated by the products investigated. It is believed that plants which are rich in a wide variety of secondary metabolites belonging to chemical classes such a tannins, terpenoids, alkaloids and polyphenols are generally superior in their antimicrobial activities (Cowan, 1999). This suggests that the strength of biological activities of a natural product is dependent on the diversity and quantity of such constituents (Geyid *et al*, 2005).

The inconsistency in the level of sensitivity could also be associated with difference in the preparation of each batch, as there are no known manufacturing standard for the product of traditional herbal remedies. This lack of standard may affect the quality of plant part used, the type of diluents/solvents use. The treatment involved in herbal remedy preparation that is, whether heating or alcohol was adopted to enhance the extraction and whether other medicinal plants were added may have a contribution. The type and level of preparation are also affected by secrecy which is an obvious characteristics of traditional medical practice in Africa. Aside from the similarly in preparation and standardization, the age of the herbal remedy also has to do with the efficacy of the herbal products.

## 5. CONCLUSION

On the basis of the results, the antibacterial activities of the herbal remedies shows varying level of sensitivity which is as a result of lack of standardized protocol for the preparation of these herbal remedies and also the age of the herbal remedy. Traditional herbal council that will act as a clearing and regulating outfit to standardized, monitor and regulate the production of herbal remedies should be instituted.

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Table 1: Mean zone inhibition (mm) of the extracts of the herbal remedies

| Product code | 1 | 2 | 3 | 4 | 5 |
|---|---|---|---|---|---|
| A | 8.63±0.03 | 9.04±0.02 | 4.69±0.01 | 2.28±0.08 | 0.00±0.00 |
| B | 16.75±0.01 | 0.35±0.01 | 0.00±0.00 | 0.00±0.00 | 0.00±0.00 |
| C | 4.56±0.02 | 1.57±0.47 | 1.09±0.01 | 0.00±0.00 | 0.00±0.00 |
| D | 20.14±0.02 | 26.45±0.06 | 6.77±0.02 | 0.72±0.08 | 0.00±0.00 |
| E | 9.47±0.01 | 6.81±0.02 | 5.31±0.13 | 0.00±0.00 | 0.00±0.00 |
| F | 13.14±003 | 8.71±0.03 | 0.97±0.04 | 10.31±0.02 | 0.00±0.00 |
| G | 10.27±0.01 | 4.68±0.00 | 11.97±0.03 | 0.00±0.00 | 3.78±0.14 |
| H | 22.68±0.02 | 13.74±0.01 | 12.16±0.02 | 25.57±0.13 | 0.00±0.00 |
| I | 18.17±0.02 | 4.86±0.01 | 0.00±0.00 | 4.66±0.04 | 0.00±0.00 |
| J | 4.20±0.01 | 12.02±0.01 | 1.13±0.01 | 0.00±0.00 | 0.00±0.00 |

Key: 1 = *Escherichia coli*, 2 = *Staphylococcus aureus,* 3 = *Streptococcus pneumoniae*, 4 = *Proteus mirabilis*, 5 = *Pseudomonas aeruginosa*

 

Table 2: Minimum inhibitory and minimum bactericidal concentration (MIC and MBC) of the herbal remedies against the test organisms

| Product code | Organisms | MIC | MBC | MIC/MBC |
|---|---|---|---|---|
| A | 1 | 25 | 25 | 1.00 |
| | 2 | 100 | >200 | <0.50 |
| | 3 | 100 | >200 | <0.50 |
| | 4 | >200 | >200 | <0.50 |
| | 5 | >200 | >200 | <0.50 |
| B | 1 | 25 | 50 | 0.50 |
| | 2 | >200 | >200 | <0.50 |
| | 3 | >200 | >200 | <0.50 |
| | 4 | >200 | >200 | <0.50 |
| | 5 | >200 | >200 | <0.50 |
| C | 1 | 100 | >200 | <0.50 |
| | 2 | 100 | >200 | <0.50 |
| | 3 | >200 | >200 | <0.50 |
| | 4 | >200 | >200 | <0.50 |
| | 5 | >200 | >200 | <0.50 |
| D | 1 | 25 | 50 | 0.50 |
| | 2 | 50 | >200 | <0.50 |
| | 3 | 50 | >200 | <0.50 |
| | 4 | >200 | >200 | <0.50 |
| | 5 | >200 | >200 | <0.50 |
| E | 1 | 100 | >200 | <0.50 |
| | 2 | 50 | 100 | 0.50 |
| | 3 | 100 | >200 | <0.50 |
| | 4 | >200 | >200 | <0.50 |
| | 5 | >200 | >200 | <0.50 |
| F | 1 | 25 | >200 | <0.50 |
| | 2 | 100 | >200 | <0.50 |
| | 3 | >200 | >200 | <0.50 |
| | 4 | 12.50 | 25 | 0.50 |
| | 5 | >200 | >200 | <0.50 |
| G | 1 | 25 | 50 | 0.50 |
| | 2 | 25 | 50 | 0.50 |
| | 3 | 25 | >200 | <0.50 |
| | 4 | 100 | >200 | <0.50 |
| | 5 | >200 | >200 | <0.50 |
| H | 1 | 6.25 | >200 | <0.50 |
| | 2 | 50 | 50 | 1.00 |
| | 3 | 50 | 100 | 0.50 |
| | 4 | 50 | >200 | <0.50 |
| | 5 | >200 | >200 | <0.50 |
| I | 1 | 25 | >200 | <0.50 |
| | 2 | 100 | >200 | <0.50 |
| | 3 | >200 | >200 | <0.50 |
| | 4 | 100 | >200 | <0.50 |
| | 5 | >200 | >200 | <0.50 |
| J | 1 | 50 | >200 | <0.50 |
| | 2 | 50 | >200 | <0.50 |
| | 3 | 50 | >200 | <0.50 |
| | 4 | >200 | >200 | <0.50 |
| | 5 | >200 | >200 | <0.50 |

Key: 1 = *Escherichia coli*, 2 = *Staphylococcus aureus,* 3 = *Streptococcus pneumoniae,* 4 = *Proteus mirabilis*, 5 = *Pseudomonas aeruginosa*