AUTHENTICATION OF THE ANTIMICROBIAL ACTIVITY OF SOME INDIGENOUS HERBAL REMEDIES USED IN THE TREATMENT OF TYPHOID AND URINARY TRACT INFECTIONS IN ANAMBRA STATE, NIGERIA

1IKEGBUNAM NM*, 10KPATA O.O, 1UGWU, M.C AND 1ESIMONE C.O
1Department of Pharmaceutical Microbiology and Biotechnology, Nnamdi Azikiwe University, P.M.B 02005 Awka, Nigeria. 
Email: nmnkechukwu@yahoo.com

ABSTRACT
This study was carried out to examine the antimicrobial activity of some indigenous aqueous herbal preparations used in the treatment of typhoid fever and urinary tract infections against some common microorganisms and to compare their antimicrobial activities with standard antibiotics. Six liquid herbal remedies indicated for the treatment of urinary tract infections (coded P1 – P3) and typhoid fever (coded P4 – P6) were purchased from various outlets of the herbal producers in Anambra state, Nigeria and screened for their activities against clinical isolates of Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa and Salmonella typhi using the agar well diffusion and agar dilution methods. The conventional antibiotics, ciprofloxacin and gentamicin were used as comparative standards. P1 was active against all tested organisms with MIC of 2.5 % for Escherichia coli, Pseudomonas aeruginosa and Salmonella and 1.25 % for Staphylococcus aureus. P2 and P3 showed activity against Staphylococcus aureus, only. P4 was effective against Salmonella typhi, Staphylococcus aureus, Escherichia coli, and Pseudomonas aeruginosa with MIC of 2.5 %, for all organismsswhile P5 and P6 had no activity against the test organisms. Ciprofloxacin showed MIC of 0.008 µg/ml for Salmonella, 0.016 µg/ml for Escherichia coli and Pseudomonas aeruginosa, and 0.002 µg/ml for Staphylococcus aureus while gentamicin showed MIC of 0.016 µg/ml for Salmonella and Escherichia coli, and 0.004 µg/ml for Pseudomonas aeruginosa and Staphylococcus aureus. Two of the herbal remedies showed inhibitory activities against the test microorganisms giving a scientific basis for the use of these herbal remedies in the treatment of urinary tract infections and typhoid. Of greater concern however is the observation that most of the herbal remedies had no activity against microorganisms contrary to their label claims. The comparison of the activities of the herbal remedies with conventional antibiotics showed that conventional antibiotics are more active than herbal preparations. It is strongly advocated that Drug Regulatory Agencies should pay high attention to the authentication of the pharmacological claims of these herbal medicines freely sold in Nigeria.

Keywords: Herbal remedies, Antimicrobial activity, Urinary tract infections, Typhoid.

INTRODUCTION
Herbal medicines have been used extensively to treat a wide range of medical conditions. Recent years have witnessed an increase in their use, but questions remain concerning their quality, safety and efficacy (QSE). The widespread availability and use of herbal medicines in today’s world indicates an increased need to evaluate objectively their effectiveness for specific conditions (Jung, 2007).

Medicinal plants have a long history of use and their use is widespread in both developing and developed countries. An estimated 80% of the world’s population still depends on traditional herbal medicines for their health security (Carter, 2001). In most African countries including Nigeria, herbal medicine is recognized as an important component of health care system, especially among rural dwellers that constitute about 70% of the population (Esimone et al., 2002). Also, the ever increasing cost of orthodox health care services coupled with the side effects of certain synthetic drug therapies, has further caused a large proportion of patients in the developing countries to resort to alternative herbal health care which they feel is natural, safer, more accessible, more economical and takes into consideration the people’s socio-cultural values (Nwogu, 1997; Carter, 2001). In the indigenous system of medicine, the plants in crude form, either fresh or dried are utilized for their curative effects against a variety of mankind’s ailments. Authentication of herbal remedies is the foundation of the safe and correct use of plant-based natural health products. Without proper authentication as a starting point, the safe use of quality products cannot be guaranteed. There is recognition within industry and government that there is a need to protect access and choice by consumers when it comes to natural health products. At the same time, consumers have a right to expect that these products can be used with confidence regarding their safety and quality (Ahmad et al., 2009). Assurances of safety, efficacy and quality of herbal medicines have been limited by lack of research methodology, inadequate evidence base for TM/CAM therapies and products, lack of international and national standards, lack of adequate regulation and registration of herbal medicines, lack of registration of TM/CAM providers and inadequate support for such research efforts (Pietroni, 1992; WHO, 1999).

The present study is in pursuance of the assessment and verification of the scientific basis of the use of some herbal medicines in Nigeria.

MATERIALS
Source of herbal products
Six (6) different liquid herbal preparations indicated for the treatment of typhoid and urinary tract infections were bought from various outlets of the herbal producers in Anambra state, Nigeria.

Table 1: Products and their therapeutic claims

<table>
<thead>
<tr>
<th>Product code</th>
<th>Product name</th>
<th>Indication(s)</th>
<th>NAFDAC Reg. No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>P1</td>
<td>CCH</td>
<td>All infections</td>
<td>Present</td>
</tr>
<tr>
<td>P2</td>
<td>Herbal antibiotic</td>
<td>Urinary tract infections</td>
<td>Absent</td>
</tr>
<tr>
<td>P3</td>
<td>Herbal mixture</td>
<td>Urinary tract infections</td>
<td>Present</td>
</tr>
<tr>
<td>P4</td>
<td>Salmoline</td>
<td>Typhoid</td>
<td>Present</td>
</tr>
<tr>
<td>P5</td>
<td>Malsol</td>
<td>Typhoid fever and malaria</td>
<td>Absent</td>
</tr>
<tr>
<td>P6</td>
<td>No name</td>
<td>Typhoid and malaria</td>
<td>Absent</td>
</tr>
</tbody>
</table>

The samples were stored in the freezer and analyzed within two weeks of purchase.
Organisms

The microbial cultures were clinical isolates of Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa and Salmonella typhi obtained from the Medical Microbiology Laboratory of Nnamdi Azikiwe University Teaching Hospital, Nnewi. They were properly identified and preserved on agar slants at 37ºC as stock.

Culture media

General purpose nutrient agar and nutrient broth (Fluka Biochemika), Sigma Aldrich Switzerland were used in this experiment. MacConkey agar (Fluka, London) was particularly employed in the confirmation of E. coli. Human blood plasma was also employed in the confirmatory test for Staphylococcus aureus.

Antibiotic disc

The antibiotic multisd used for the sensitivity test was Optudisc® (Optum laboratories, Nig. Ltd.) containing: OFX-Ofloxacin (10mcg); PEX-Pefloxaxine (10mcg); CPX-Ciprofloxacin (10mcg); AU-Augmentin (30mcg); CN-Gentamycin (10mcg); S-Streptomycin (30mcg); CEP-Ceporex (10mcg); NA-Nalidixic acid (30mcg); SXT-Septin (30mcg); PN-Ampicillin (30mcg).

METHODS

Standardization of inoculums

Inoculums of the organism growing as pure culture in the nutrient agar slants were suspended in sterile water. The opacity of the bacterial dilution was adjusted to get a standard suspension using the 0.5 McFarland Standard as a comparative standard.

Antibiotic Sensitivity Testing

About 0.1ml of the overnight broth culture of Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa and Salmonella typhi was taken and aseptically transferred into labelled sterile Petri dishes. Then 15ml of molten sterile nutrient agar was poured into the seeded Petri dishes and swirled to distribute the medium homogenously. After solidification, holes were made aseptically with a 6mm sterile cork borer and 0.1ml of the test solution of different concentrations was introduced into the wells. The agents were allowed to diffuse into the medium and then incubated aerobically for 24 hours at 37ºC.

One well containing water served as control in each plate. The plates were examined for zones of inhibition, which indicate the degree of susceptibility of the test organisms. The antimicrobial activity of the various agents was measured with a metre rule and compared with the control well (containing water).

Determination of Minimum Inhibitory Concentration (MIC)

MIC of the aqueous herbal preparations which showed significant activity against the test microorganisms was determined by preparing two-fold serial dilutions to concentrations of 100%, 50%, 25%, 12.5%, 6.25%, and 3.12%. 1ml of each concentration was introduced into sterile Petri dishes, and then 19ml of sterile nutrient agar was added and mixed for homogeneity. After the agar had solidified, inoculums of the test organisms were streaked on the surface of each plate. The plates were incubated aerobically at 37 ºC for 24 hours. The standard antibiotic drugs gentamicin sulphate and ciprofloxacin were also screened under similar conditions for comparison. Two control plates were maintained for each test batch. These included antibiotic control (plate containing agents and the growth medium without the inoculums) and organism control (plate containing the growth medium and the inoculums). The lowest concentration (higher dilution) of the agent that produced no visible bacterial growth when compared with the control plate was regarded as the MIC.

RESULTS

The results of this experiment are presented in Tables 2-4.

Generally there were inhibitions of growth of the test organisms as indicated on culture plates by cleared zone. The activity of the herbal remedies on the test organisms was not uniform (Table 2) In all cases S. aureus showed high sensitivity (38 mm zone of inhibition) followed by E. coli (20 mm), P. aeruginosa (16 mm) and S. typhi (14mm).

It was observed that all test organisms were susceptible to Ciprofloxacin, Streptomycin and Cotrimoxazole while Pseudomonas aeruginosa and Escherichia coli were resistant to Ampicillin with P. aeruginosa alone being resistant to Ceporex (Table 3).

Table 2 shows Staphylococcus aureus having the least MIC values (1.25%/v/v and 0.002µg/ml) as most sensitive to both herbal products and standard antibiotics, respectively.

---

**Table 2: Antibacterial activity of herbal preparations on the test organisms**

<table>
<thead>
<tr>
<th>Test Orgs</th>
<th>Diameter of zone of inhibition (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Product 1</td>
</tr>
<tr>
<td>S. typhi</td>
<td>20</td>
</tr>
<tr>
<td>E. coli</td>
<td>16</td>
</tr>
<tr>
<td>P. aeruginosa</td>
<td>18</td>
</tr>
<tr>
<td>S. aureus</td>
<td>38</td>
</tr>
</tbody>
</table>

_ indicates no zone of inhibition

**Table 3: Result of sensitivity of test microorganisms to some standard antibiotics**

<table>
<thead>
<tr>
<th>Organ</th>
<th>Diameter of zone of inhibition (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CPX</td>
</tr>
<tr>
<td>Salmonella typhi</td>
<td>40</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>35</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa</td>
<td>34</td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>37</td>
</tr>
</tbody>
</table>

CPX - Ciprofloxacin, S - Streptomycin, SXT - Cotrimoxazole, CEP - Ceporex, PN – Ampicillin

_ indicates no zone of inhibition
The product also contains a preliminary report of the efficacy of some herbal products and logical claims of these herbal medicines freely sold. Rational use of pharmaceutical drugs is key. It is strongly advocated that Drug Regulatory Agencies should pay high attention to the authentication of the pharmacological claims of these herbal medicines freely sold in Nigeria.

REFERENCES