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Research Article

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

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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 %$^{v}/_{v}$ for *Escherichia coli, Pseudomonas aeruginosa* and *Salmonella* and 1.25 %$^{v}/_{v}$ 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 %$^{v}/_{v}$, for all organismswhile 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 (Nwaogu, 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

Product code	Product name	Indication(s)	NAFDAC Reg. No.
P1	CCH	All infections	Present
P2	Aherbal antibiotic	Urinary tract infections	Absent
P3	Herbal mixture	Urinary tract infections	Present
P4	Salmoline	Typhoid	Present
P5	Malsol	Typhoid fever and malaria	Absent
P6	No name	Typhoid and malaria	Absent

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 37^{0}C 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 multidisc used for the sensitivity test was Optudisc® (Optun laboratories, Nig. Ltd.) containing: OFX-Ofloxacin (10mcg); PEX-Pefloxacine (10mcg); CPX-Ciprofloxacin (10mcg); AU-Augmentin (30mcg); CN-Gentamycin (10mcg); S-Streptomycin (30mcg); CEP-Ceporex (10mcg); NA-Nalidixic acid (30mcg); SXT-Septrin (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, the antibiotic multidisc (Optun disk, Nig.) was aseptically placed on the surface of the solidified nutrient agar and then incubated aerobically for 24 hours at 37^0 C. A seeded nutrient agar plate without a multidisc was used as control. The plates were examined for zones of inhibition, which indicate the degree of susceptibility of the test organisms to the various antibiotics present in the multidisc. The antimicrobial activity of the various agents was measured with a metre rule as inhibition zone diameter.

Agar well diffusion method

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 37^0 C.

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 ^{0}C 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 4 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

Test Orgs	Diameter of zone of inhibition (mm)					
	Product 1	Product 2	Product 3	Product 4	Product 5	Product 6
S. typhi	20	_	_	14	_	_
E. coli	16	_	_	20	_	_
P. aeruginosa	18	_	_	16	_	_
S. aureus	38	16	20	33	_	_

_ indicates no zone of inhibition

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

	Diameter of zone of inhibition (mm)				
	CPX	S	SXT	CEP	PN
Salmonella typhi	40	36	31	28	30
Escherichia coli	35	30	18	26	_
Pseudomonas aeruginosa	34	29	19	_	_
Staphylococcus aureus	37	27	27	20	27

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

_ indicates no zone of inhibition

Table 4: The MIC of test products and standard antibiotics against test organisms

Test Organisms	MIC of Products (% $^{v}/_{v}$)		MIC of Antibiotics (µg/ml)	
	Prdt 1	Prdt 4	GEN	CPX
Salmonella typhi	2.5	2.5	0.016	0.008
Escherichia coli	2.5	2.5	0.016	0.016
Pseudomonas aeruginosa	2.5	2.5	0.004	0.016
Staphylococcus aureus	1.25	2.5	0.004	0.002

GEN - Gentamicin, CPX – Ciprofloxacin

DISCUSSION

The profiles of herbal remedies used in this study are shown in Table 1. Of all three herbal remedies indicated for the treatment of urinary tract infections, only one (P1) had activity against all the test organisms. The other two showed activity against *Staphylococcus aureus* only;therefore, they may be recommended for infections caused specifically by *S. aureus.*

Only one (P4), out of the three herbal remedies indicated for the treatment of typhoid showed activity against *Salmonella typhi,* thus its use in the treatment of typhoid can be justified.The product also showed broad spectrum of activity against the other test organisms, *E. coli, S. aureus* and *P. aeruginosa*, so may be useful in the treatment of conditions caused by these organisms. The other two products (P5 & P6) had no activity against the test organisms. Failure of some of these herbal remedies to exert antibacterial effect on test organisms may not be enough to conclude that they do not contain substances that can exert antibacterial activity against the test organisms because the potency of these herbal remedies depends on their method of production (Anibijuwon et al, 2010). Research has shown that the age of plant when harvested and the season of harvest determine the amount of the active constituents and since the active ingredients of plants can vary in quality and quantity from season to season, their efficacy can thus be affected (Sofowora, 1982).

The antibiotics had inhibitory activities at minute concentrations when compared with that of the herbal products (Table 4). The comparison of the activity of the herbal remedies with conventional antibiotics showed that conventional antibiotics are more active than herbal preparations as confirmed by other workers (Anibijuwon et al, 2010).

The World Health Organization has been advocating the need for orthodox medical practitioners to interact with traditional herbal healers with a view to identifying and exploiting aspects that provide safe and effective remedies for ailments of both microbial and non-microbial organisms (WHO 1978). No previous scientific study has been carried out on the herbal preparations used in this study but previous work by Kilani et al (2007) on the antifungal activity of a herbal decoction used by south western Nigeria traditional healers in the treatment of superficial mycoses and related infectious diseases showed that the decoction had activity against the clinical isolates (*Candida albican, Trichophyton rubrum* and *Microsporium* sp.) it was tested against.

This study presents a preliminary report of the efficacy of some indigenous herbal preparations used in the treatment of typhoid and urinary tract infections as demonstrated by *in vitro* activities against the clinical isolates tested.

CONCLUSION

This study offers a scientific basis for the use of some herbal remedies in the treatment of urinary tract infections and typhoid.

The use of herbal medicinal products is on the rise and so there is an increased requirement to monitor and assure their QSE. Improved regulations that recognise the specific requirements and characteristics of herbal medicines compared to conventional 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.

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