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Antimicrobial activities of some herbal anti-infectives manufactured and marketed in South-East Nigeria

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The aim of this study was to evaluate the anti-microbial activities of some liquid herbal anti-infectives manufactured and marketed in South-Eastern Nigeria and determine the extent of their microbial contamination. Twenty samples were randomly collected from herbal shops in the five states that make up the South-East Nigeria. Antibacterial activities of the herbal preparations were evaluated using agar-well-diffusion method. The samples that showed significant antibacterial activity against the test organisms were further subjected to cell killing rate test. Maximum inhibitory dilutions of the active compounds were obtained and their *in vitro* anti-infective activity against multidrug resistance *Staphylococcus aureus* (MRSA) and Extended Spectrum β-Lactamase (ESBL) organisms were evaluated also. Eight (40%) of the product showed some antibacterial activity and none have antifungal activity against the test fungi. Kill kinetic experiment showed that some products have some activity against the test bacteria. One of the products showed antibacterial activity against MRSA. Comparison of the antibacterial activity of the products and conventional antibiotics showed that there was no significant difference among the microorganism to the antibiotics (F = 0.498, P = 0.686) and herbal anti-infectives (F = 0.477, P = 0.700). Only 40% of the 20 products have some antibacterial activity but none have anti-fungal activity. All were heavily contaminated with microorganisms. Current good manufacturing practice may not have been applied in their manufacture.

Key words: Herbal anti-infectives, antibacterial activity, South-East Nigeria, liquid preparations.

INTRODUCTION

Health for all is a dream and a goal which humanity at large shares and strives for but is clear that modern pharmaceuticals are and will remain out of reach for a large proportion of the human population for the foreseeable future (Mosihuzzaman and Choudary, 2008). This gap has created the need for the use of alternative and traditional medicines, largely herbal in nature, to solve human health need. Alternative medicines, such as herbal medicines, are gaining popularity because of typically low side-effect profiles (Wilt et al., 2000), low cost (Vanderhoof, 2001), and a high level of acceptance by patients. Some managed care organizations now offer these therapies as an expanded benefit (Langyan and Ahuja, 2005). In Africa, traditional medicine has always been a part of the culture even though this form of medicine is not as well organized as, for example, in India and China (Ogunshe et al., 2006). Herbal medicine has become a popular form of healthcare at least in African and Asian countries being intertwined with modern medicine (Eisenberg et al., 1998; Esimone et al.,

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2002). The use of herbal medicine has always been part of human culture, as some plants possess important therapeutic properties (Barkatullah et al., 2013; Selim et al., 2013). The ideas that certain plants had healing potentials and contain antimicrobial principles were well accepted long before mankind discovered the existence of microbes (Rios and Recio, 2005). Traditional herbalists in Nigeria use various herbal preparations to treat various types of ailments, including diarrhea, urinary tract infections, typhoid fever and skin diseases (Sofowora, 1993).

In developing countries, "traditional medical practice is often viewed as an integral part of their culture" (Kunle *et al.,* 2012; Evans, 1996), traditional medical practice is often viewed as an integral part of their culture. Although it is generally believed that most herbal preparations are safe for consumption, some herbs contain biologically active substances that can be toxic or at least have undesirable side effects (Evans, 1996). There is no effective machinery to regulate manufacturing practices and quality standards (Kunle et al., 2012). Given the variable nature of products of plant origin, ensuring consistent quality of their products is vital for the survival and success of the industry (Bauer, 1998). In Nigeria, there appears to be an overwhelming increase in the public awareness and usage of herbal medical products in the treatments and/or prevention of diseases (Okunlola et al., 2007). With this increased usage, the safety, efficacy and quality of these medicines have been an important concern for health authorities and health professionals (Oluyege and Adelabu, 2010). Many of these products have bogus claims on their labels and these claims may have also led to the increase usage of the products. Advertising in various forms by the herbal practitioners is unparalleled in Nigeria. People now attend hospitals as often as they go to herbalists (Okunade, 2001).

This study was therefore meant to examine the antibacterial properties of some liquid herbal anti-infectives produced and marketed in South-East Nigeria and to estimate their level of contamination.

MATERIALS AND METHODS

The materials used include: nutrient agar media (Lab M, UK), MacConkey agar, (Fluka, UK), Xylose Lysine Deoxycholate Agar, Urease broth, Citrate agar, Saubouraud Dextrose agar (BIOTECH Laboratories LTD, UK), Mueller Hinton agar media (Oxoid, UK) and Peptone water. All the media were prepared aseptically following the manufacturers' instructions.

Study area

The study sampled herbal anti-infectives from South-Eastern Nigeria, West Africa. South-Eastern Nigeria comprises five states which include: Abia, Anambra, Ebonyi, Enugu and Imo states.

Samples collection

A total of twenty (20) different liquid herbal anti-infectives were purchased randomly from identified herbal shops and retail outlets across the South-Eastern states of Nigeria. The samples as shown in Table 1 were kept at room temperature (28±2°C) and used within two weeks of collection.

Test organisms

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

Identification and characterization of the bacteria isolates

Wire-loopful quantities of the products were stricken onto MacConkey agar, Blood agar, and Sabouraud agar. The plates, incubated aerobically at 37°C for 24 to 48 h, were examined for growth and biochemical tests carried out according to the methods described by Monica (2002) for proper identification of the organisms isolated.

Agar well diffusion method

About 0.1 ml of 0.5 Mcfarland standards of clinical isolates of *S. aureus, E. coli, P. aeruginosa, S. typhi* and *C. albicans* was taken and aseptically transferred into labeled sterile Petri dishes. Then 20 ml of molten sterile nutrient agar was poured into the seeded Petri dishes and swirled to distribute the medium homogenously. After solidification, holes of dept 3.5 mm were made aseptically with a 6 mm sterile cork borer and sealed. The stock and 2 fold dilution of the liquid herbal products (volume = 0.1 ml) were introduced into separate wells and allowed to diffuse into the medium. The whole set up was then incubated aerobically for 18 to 24 h at 37°C. One well containing sterile water served as control and another containing amoxicillin/clavulanic acid (AMC, 20/10 µg) served as a positive control in each plate. The antimicrobial activity of the various agents was determined by measurement of the inhibition zone diameter using a meter rule and compared with the control well (containing water).

Determination of ant-bactericidal activity of the herbal anti-infectives using kill kinetic method

Nutrient broth of volume (5 ml) was dispensed into six culture tubes provided for each indicator bacteria and was sterilized. These were labeled 0 to 5 h where 0 h serves as control. One milliliter of four different herbal anti-infectives (product 2, 3, 7, 11) that showed significant antibacterial activity by the agar diffusion method were added to the culture tubes containing the indicator bacteria. The suspension of the indicator bacteria and herbal medicine were thoroughly mixed and held at room temperature (28±2°C). Anti-bactericidal activity was determined by plating 0.1 ml of the suspension at hourly interval for up to 5 h. The plates were incubated and the colony forming units were counted

Determination of maximum inhibitory dilution (MID)

The MID of the aqueous herbal preparations which showed by the micro-broth dilution method. Serial dilutions of herbal anti-infectives in dilutions of 5:0, 4:1, 3:2, 2:3, 1:4 and 0:5 of the broth and herbal products, respectively were prepared in sterile test tubes. Standard

Table 1. Some brands of herbal anti-infectives marketed in South-East Nigeria.

Product code	NAFDAC Reg. NO.	Contents	Therapeutic claim
1	04-8618L	*Carica papaya*, *Magnifera indica*, Newbouldlia leaves, *Azadricha indica*, *Jaminum officionili*, *Aloe barbedensis*, ginseng, treated water 60cl.	Antibacterial, antimalarial, antirheumatic, infertility, antiviral
2	1295	38 African roots, herbs, fruits, barks plus ginseng, aloe vera and garlic.	Antibacterial, antirheumatic, antifungal and antiviral
3	-	60% Herbs, 25% flower, 10% leaves, and 5% roots.	Antibacterial, Antirheumatic, antifungal, earlier menopause, painful and irregular menstruation
4	A7-0280L	Aloe vera plus 31 roots and herbs, fruits and barks	Antibacterial and antifungal
5	-	Water, herbs, root and fruits	Internal heat, pile, antibacterial, antimalarials, anti-parasitic and reduces blood sugar
6	NUOMHP NOHerbal:0 9840	-	Antibacterial, treatment of all kinds of eye infections
7	No.A7-0736L	*Nauclea diiderchi* 10%, *Hippocrates pallens* 20%, *Alluim sativum* 12.5%, *Cochios permum pianchoni* 5.5%, *Uvaria chame* 5%, *Punica granatum* 47%	Antibacterial, antimalarials
8	No	Aloe vera 40%, Olong tea 20%, flower and roots 40%, saracin.	Antibacterial
9	No	Aloe vera	Antibacterial anti-malarial, HBP, cough antirheumatism, etc.
10	No.A7-0220L	Aloe vera, flowers, and fruits seed barks.	Antibacterial, hypertension, antiviral, fibroid, stroke.
11	No	Honey (natural), lime juice, *Zingiber officinale*, and herbal seeds and roots.	Antibacterial and asthmatic cough.
12	No	-	Anti-bacterial antiviral, diabetes, reduces cholesterol.
13	No	25 different types of roots, herbs, seeds and flowers.	Anti-bacterial, anti-malaria, antirheumatic. Antifungal
14	No	Herbs, water, root and fruits.	Antibacterial, Internal heat, pile, Antiviral, Antirheumatic, Antifungal, Antiparatic.
15	No.Al-0240L	*Mangifera indica, Carica papaya* leaves*, Psidium guajava, Masularia acuminate* root, breadfruit bark, citrus lemon leaves, *Zingiber officinale* root*, Cymbopogon* spp.	Antibacterial, Treatment and prevention of toothache.
16	No	Awapa bark, white lotus, Golden seal, mahogany, Ukor root, Aloe barbaders, Mistletoe, Osisika Aguru, Uda roots, Uvuru ilu, lemon grass.	Antibacterial, antirheumatic and arthritis, venereal diseases.
17	No.A7-1482L	Aloe vera, Cadeperi salt, lime	Antibacterial, treatment and prevention of toothache.
18	No.A7-0912L	Lymbopogon citrates, *Carica papaya* leaves, *Magnifera indica*, bark, Treculia Africana, Citrus, Limonia, *Psiduim guajava, Zingibar officinale* root, *Alluim sativum.*	Antibacterial, antirheumatism, reduces sugar and cholesterol.
19	No.41563	Natural roots and barks.	Antibacterial, antiviral, purifies blood, detoxifies toxins, builds immune system, stops dizziness, weakness.
20	No	Nuclealatifolia, *Allium sativum*, Aloe vera bitter, Chick weed, *Preclina nitida, Hibiscus sabdrrifa*, Aqua, ethanol.	Antibacterial, antiparasitic, ulcer, constipation, fibroid, internal heat heart burn and diabetes

inoculums of the test microorganisms equivalent to 0.5 McFarland were prepared and 0.1 ml of the standard inoculum introduced into test tubes containing the dilutions of the herbal products. The tubes were incubated aerobically at 37°C for 24 h. After 24 h a loopful of the different concentration were taken from the tubes and streaked on agar plates, which were also incubated at 37°C for 24 h after which the presence or absence of growth was observed and matched with the particular concentration. The highest dilution (lowest Concentration) of the agent that produced no visible bacterial growth when compared with the control plate was regarded as the MID.

Determination of the antimicrobial activity of the products against MRSA and ESBL- expressing *E. coli* and *Klebsiella* species

About 0.1 ml of the standardized suspension (equivalent to 0.5 McFarland) of MRSA, ESBL expressing *Escherichia coli* and *Klebsiella* were obtained from the stock available in pharmaceutical and biotechnology lab, Faculty of Pharmacy, Nnamdi Azikiwe University, Agulu campus and aseptically transferred into labeled sterile Petri dishes. Then 20 ml 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 6 mm sterile cork borer and 1 ml of the test solution of different concentrations introduced into the wells. The agents were allowed to diffuse into the medium and then incubated aerobically for 18 to 24 h at 37°C. 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 meter rule and compared with the control well (containing water).

Determination of the level of microbial contamination of the products

Exactly 1 ml of each herbal anti-infectives was aseptically transferred into a corresponding sterile test tube containing 9 ml of sterile distilled water and ten-fold serial dilution was carried out. Using the Pour plate technique, 1 ml of dilutions 10^{-1}, 10^{-2} 10^{-3}, 10^{-4}, and 10^{-5} were transferred to clean sterilized Petri dishes and mixed with 20 ml of sterile molten nutrient agar and Sabouraud Dextrose agar which was cooled to 45°C for bacteria and fungi, respectively. These were done in triplicates and the plates were allowed to set and then incubated at 37°C for bacterial counts from 18 to 24 h, and 20 to 27°C for fungal count for 72 to 168 h. All counts were expressed in CFU/ml.

Statistical analyses

Analysis of variance (ANOVA) was performed to determine statistical significant differences in inhibition zone diameter (IZD) amongst the herbal products and the conventional antibiotics. The level of significance was set at 0.05.

RESULTS

Table 1 shows the different liquid herbal anti-infectives that were evaluated in this work. All the products (100%) had antibacterial claims, 6 (30%) claimed to have antifungal activity while 13 (65%) claimed to be effective for the management of non-infectious diseases/ conditions. None of the products investigated demonstrated any antifungal activity and only 40% of the products showed some antibacterial activity.

The antimicrobial activity of the herbal anti-infectives was evaluated against selected pathogenic bacteria and fungi as presented in Table 2 using agar well diffusion method. The table revealed that none of the herbal samples has antifungal effect, 3 out of the 20 inhibited the growth of *S. aureus,* with product 2 stock having the highest zone of inhibition (26 mm) and product 11 stock having the least (16 mm). Three of the herbal product inhibited the growth of *Salmonella typhi* with product 3 and 17 having a higher zone of inhibition of 12 mm than product 2 which has 10 mm IZD. The growth of *P. aeruginosa* was inhibited by 5 products. Product 2 had the highest IZD of 22 mm while products 5 and 9 have the least which is 10 mm. Growth of *E. coli* was inhibited by two products; product 7 (IZD of 25 mm) and product 11 (IZD of 14 mm).

The bactericidal activity of the products (2, 3, 7 and 11) that showed significant antibacterial against at least two of the target bacteria was characterized using killing rate kinetics method (Table 3). The MID of the products that showed significant antibacterial activity against the test organisms is given in Table 4 and the activity of the herbal products against MRSA and ESBL expressing *E. coli* and *Klebsiella* is shown in Table 5. Table 6 shows the comparison of IZD of the herbal anti-infectives and conventional antibiotics. Table 7 shows the level of microbial contaminations of the products.

DISCUSSION

The herbal medicinal products selected for this study were all liquid dosage forms. Products 2, 7, 10, 12, and 17 were purchased from Aba in Abia state, products 1, 5, 6, 9, and 16 were from Anambra state, products 3, 4, 13, 18, 19, and 20 were from Ebonyi and Enugu States while Products 8, 14, and 15 were from Owerri in Imo State. All the samples were within their shelf-life at the time of investigation. Ten (50%) of the products had NAFDAC registration number while the other 10 (50%) were not registered by the agency. This is contrary to the laws governing the manufacture, advertisement, sale and distribution of herbal medicinal products and indeed all foods and drugs in Nigeria which forbids such when the products are not properly registered by NAFDAC (HMRP, 2004). The European Agency for the Evaluations of Medicinal Products (EAEMP) and WHO have stated that the quality of the herbal drug should be given as a range corresponding to a defined quantity of constituents with known therapeutic activity and if constituents responsible for therapeutic activity are unknown, the quantity of the whole herbal drug preparation should be given (EMEA, 2002; WHO, 1996a). They also stipulated that the dosage

Table 2. Products' antimicrobial activity on the test organisms.

Product	IZD (mm)									
	E. coli		*P. aeruginosa*		*S. aureus*		*S. typhi*		*C. albicans*	
	Stock	Dilution	Stock	Dilution	Stock	Dilution	Stock	Dilution	Stock	Dilution
Product 1	-	-	18	18	-	-	-	-	-	-
Product 2	-	-	22	20	26	22	10	10	-	-
Product 3	-	-	16	16	-	-	12	12	-	-
Product 4	-	-	-	-	-	-	-	-	-	-
Product 5	-	-	10	10	-	-	-	-	-	-
Product 6	-	-	-	-	-	-	-	-	-	-
Product 7	25	18	-	-	18	18	-	-	-	-
Product 8	-	-	-	-	-	-	-	-	-	-
Product 9	-	-	10	10	-	-	-	-	-	-
Product 10	-	-	-	-	-	-	-	-	-	-
Product 11	14	12	-	-	16	12	-	-	-	-
Product 12	-	-	-	-	-	-	-	-	-	-
Product 13	-	-	-	-	-	-	-	-	-	-
Product 14	-	-	-	-	-	-	-	-	-	-
Product 15	-	-	-	-	-	-	-	-	-	-
Product 16	-	-	-	-	-	-	-	-	-	-
Product 17	-	-	-	-	-	-	12	12	-	-
Product 18	-	-	-	-	-	-	-	-	-	-
Product 19	-	-	-	-	-	-	-	-	-	-
Product 20	-	-	-	-	-	-	-	-	-	-

S = Stock Concentration, D = Diluted Concentration (2-fold), - = No inhibition.

Table 3. Anti-bacterial activity of the products with significant activity using kill kinetic method.

Time (h)	Bacterial load (CFU/ml)									
	S. typhi			*S. aureus*			*E. coli*		*P. aeruginosa*	
	Prod. 2	Prod. 3	Prod. 7	Prod. 2	Prod. 7	Prod.11	Prod. 7	Prod.11	Prod. 2	Prod. 3
0	221	221	248	49	41	41	44	19	77	112
1	209	202	236	37	19	29	24	22	56	95
2	204	185	204	26	38	26	3	15	48	81
3	180	179	146	20	56	20	0	12	44	66
4	170	172	87	17	10	17	0	0	29	51
5	162	162	51	0	0	10	0	0	19	39

Prod.: Product.

Table 4. MID of the products with significant antibacterial activity.

Test organism	MID of the products (% v/v)			
	Product 2	Product 3	Product 7	Product 11
S. typhi	66.6	25	66.6	-
P. aeruginosa	66.6	66.6	-	-
S. aureus	150	-	66.6	150
E. coli	-	-	66.6	150

form, therapeutic indications and expiry dates should be stated. However, 3 (15%) of the products did not have their content stated even though therapeutic claims were indicated either on the container or in the leaflet insert.

Table 5. Antibiotic activity of the products against MRSA and ESBL-Expressing Organisms.

Product	MRSA 1		MRSA 2		ESBL (*E. coli*)		ESBL (*Klebsiella*)	
	Stock	Dilution	Stock	Dilution	Stock	Dilution	Stock	Dilution
2	-	-	-	-	-	-	-	-
3	-	-	-	-	-	-	-	-
7	30	27	13	13	-	-	-	-
11	-	-	-	-	-	-	-	-

MRSA: Methicillin Resistant *Staphylococcus aureus*; ESBL: Extended Spectrum Beta-Lactamase; Dilution: Two-fold dilution; -: No inhibition.

Table 6. Inhibition Zone Diameter (mm) of the products and conventional antibiotics.

Test organism	P 2	P 3	P 5	P 7	P 11	OFX	CIP	TE	GN	CRO
S. typhi	10	26	-	24	-	15	32	14	26	-
P. aeruginosa	22	16	10	-	-	-	17	-	20	16
S. aureus	26	-	-	18	16	25	20	8	-	13
E. coli	-	-	-	18	14	-	15	-	16	-

P: Product; OFX: ofloxacin; CIP: ciprofloxacin; CAZ: ceftazidime; TE: tetracycline; AMP: ampicillin; SXT: sulfamethoxazole-trimethoprim; GN: gentamicin; CTX: cefotaxime; CRO: ceftriaxone; AMC: amoxycillin-clavulanic acid.

Table 7. Level of microbial contamination of the products.

Product code	Bacterial count (CFU/ml)				Fungal count (CFU/ml)			
	Plate1	Plate 2	Plate 3	$\dot{X}$±SEM	Plate 1	Plate 2	Plate 3	$\dot{X}$±SEM
1	28	35	23	2.9×10^{3} ±3.48	5	7	3	5×10^{4}±1.15
2	27	38	30	3.2×10^{2}±3.28	4	3	5	4×10^{4}±0.58
3	36	48	50	4.5×10^{4}±4.37	9	4	2	5×10^{5}±2.08
4	35	40	42	3.9×10^{3}±2.08	7	12	4	8×10^{3}±2.33
5	90	73	67	7.7×10^{2}±6.89	0	0	0	- -
6	28	47	32	3.6×10^{3}±5.78	16	2	1	6×10^{2}±4.84
7	7	7	7	7×10^{1}±0.00	46	20	26	3.1×10^{3} ±7.86
8	4	51	10	2.2×10^{4}±14.77	30	32	28	3.0×10^{3}±1.15
9	25	92	15	4.4×10^{3}±24.17	35	18	17	2.3×10^{2}±5.84
10	76	67	60	6.8×10^{4}±4.63	25	25	20	2.3×10^{4}±1.67
11	250	250	200	2.33×10^{6}±16.67	30	25	5	2.0×10^{3}±7.64
12	116	120	100	1.12×10^{5}±6.11	94	90	94	9.4×10^{5}±1.33
13	48	35	40	4.1×10^{5}±3.79	30	38	40	3.6×10^{3}±3.06
14	93	62	70	7.5×10^{3}±9.29	30	36	32	3.3×10^{4}±1.76
15	41	45	40	4.2×10^{3}±1.53	35	30	15	2.7×10^{5}±6.01
16	64	55	68	6.2×10^{3}±3.84	40	41	39	4.0×10^{5}±0.58
17	48	50	45	4.8×10^{3}±1.45	50	49	51	5.0×10^{5}±0.58
18	109	100	98	1.02×10^{6}±3.38	10	15	11	1.2×10^{5}±1.53
19	15	20	30	2.2×10^{2}±4.41	55	50	56	5.4×10^{4}±1.86
20	46	40	38	4.1×10^{4}±2.40	12	12	12	1.2×10^{2}±0.00

All the products (100%) had antibacterial claims, 6 (30%) claimed to have antifungal activity while 13 (65%) claimed to be effective for the management of non-infectious diseases/conditions. Medicinal products designed for the purpose of chemotherapeutic and pharmacological benefits should be effective against the target medical condition.

The evaluation of the antimicrobial activity of the products against test organisms (*S. aureus, E. coli, S. typhi, P. aeruginosa* and *C. albicans*) (Table 2) showed

that only 8 (40%) of the herbal products (products 1, 2, 3, 5, 7, 9, 11, and 17) have some antibacterial activity. Four out of the eight products showed significant activity. None of the products showed antifungal activity against the test fungi organism even though six (30%) of the products (2, 3, 4, 10, 13, and 14) claimed to have antifungal activity. The poor antimicrobial activity as shown by the herbal products against the test organisms could be attributed to the fact that the products were contaminated as shown in Table 7. The presence of microbial contaminants in non-sterile pharmaceutical products can reduce or even inactivate the therapeutic activity of the product and has the potential to adversely affect patients taking the medicines (Nakajima et al., 2005; Okunlola et al., 2007). Apart from possible microbial degradation of the active constituents contained in the herbal preparations, the presence of these contaminating microorganisms could constitute a source of infection and serious health risk to the consumers of the herbal preparations who were probably already overwhelmed by the serious medical conditions for which the herbal drugs were initially indicated (Mangram et al., 1999; Bowler et al., 2001). Spoilage of medicines involve initial or pioneer invading biodegrading microorganisms, which prepare the way for later invaders that biodegrade complex nutrient, thus altering the surrounding pH and increasing moisture content (Omwuliri and Wonang, 2005). On the other hand, the anti-microbial claims of the products may not be true.

Further evaluation on the bactericidal action of the herbal products with significant antibacterial activity was done using kill kinetic method. The herbal preparations showed bactericidal effect on the indicator bacteria as bacterial load decreased from the first hour to the fifth hour (Table 3). Product 7 seems to have erratic effect on *S. typhi*. The organism was not very susceptible to the products generally. *E. coli* was found to be susceptible to antibacterial actions of herbal preparations 7 and 11, *S. aureus* was found to be susceptible to the antibacterial actions of products 2 and 7 but less to product 11. Susceptibility of *P. aeruginosa* to the products 2 and 3 was also observed as the products were able to reduce the bacteria load significantly although more time was observably needed to clear the organisms. The MID of the herbal products that showed significant antibacterial activity was also evaluated (Table 4), and the result showed that at higher dilutions, the products have little or no antibacterial activity. Evaluation of the actions of the products against MRSA and ESBL expressing *E. coli* and *Klebsiella* spp. (Table 5) revealed that only one of the products (Product 7) showed activity against MRSA and none had activity against ESBL-expressing organisms. This shows that in cases of infections caused by these organisms, the products are powerless.

In the present study, it was observed that herbal products were grossly contaminated by fungal and bacterial agents, with the exception of one that was not contaminated with fungi (Product 5) (Table 7). Contamination by microorganisms is influenced by the environment, improper handling and storage of medicinal plants (Idu et al., 2010; Oleghe et al., 2011). Herbal medicinal plants usually contain bacteria and molds from soil and atmosphere. Microorganisms are everywhere. This can be supported by the results of the present research as the herbal products from all the five states were all contaminated. One of the major shortcomings of herbal preparations in developing countries is the unhygienic condition under which they are produced *(*Frazier and Westhoff*,* 2003).

From the ANOVA, the comparison of the herbal products with conventional antibiotic shows no significant difference. This suggests that both the herbal products and the antibiotics have the potential to produce anti-bacterial effect (Table 6), but aseptic techniques may not have been applied during the manufacture of the products. Some studies on the sterility of the manufacturing environment of these companies are hereby suggested.

Conclusion

Only 40% of the 20 products have some antibacterial activity, but none have anti-fungal activity. All were heavily contaminated with microorganisms. Current good manufacturing practice may not have been applied in their manufacture.

ABBREVIATIONS

EAEMP, The European Agency for the Evaluations of Medicinal Products; **EMEA,** European Medicines Evaluation Agency; **NAFDAC,** National Agency for Food Drug Administration and Control; **HMRP,** Herbal Medicines and Related Products; **MRSA,** methicillin resistant *Staphylococcus aureus*; **ESBL,** extended spectrum beta-Lactamase; **HMPWP,** Herbal Medicinal Products Working Party.

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