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VALIDATION OF ANTIBACTERIAL CLAIMS OF SOME HERBAL MEDICINAL PRODUCTS SOLD IN OTUKPO, BENUE STATE.

ELLA[1], A. B., ELLA[2], F. A., ADULUGBA[1], A. O. AND ORGAH[1], V. O.

[1]Department of Science Laboratory Technology, Benue State Polytechnic, Ugbokolo
[2]Department of Biology Education, Federal College of Education (Technical), Umuneze, Anambra State

Corresponding authors email: ellaadakole02@gmail.com

ABSTRACT
The study is aimed at validating antibacterial claims of some herbal products sold in Otukpo, Benue State. The test organisms include: *Staphylococcus aureus, Salmonella typhi, Escherichia coli*, and *Proteus vulgaris*, which were obtained from Medical Laboratory unit of General Hospital Otukpo and were confirmed by sub-culturing on appropriate selective media and the use of biochemical tests in the Microbiology Laboratory, Department of Science Laboratory, Benue State Polytechnic, Ugbokolo. Ciprofloxacin was used as a positive and negative control. The degree of susceptibility of the test isolates to each herbal product was determined by measuring the diameter of the zones of inhibition. Product Code I had the highest zone of inhibition of 36 mm against *Escherichia coli*, followed by Product Code F with zone of inhibition of 32 mm, while the least sensitive against *Escherichia coli* was Product Code D with zone of inhibition of 17 mm; all the herbal products where resistant to *Staphylococcus aureus.* Against *Escherichia coli*, the MIC and MBC values for Product Code F were 75 mg/ml and 100 mg/ml, respectively; while for *Salmonella typhi*, the MIC and MBC values for Product Code G were 28 mg/ml and 70 mg/ml, respectively. The overall result showed that most of the antibacterial claims by the herbal producers were not true as validated in this present work.

Keywords: Validation, Bacterial isolates, Antibacterial, Claims and Medicinal products

INTRODUCTION

Traditional medicine is an empirical study, based on traditional theory, pathology, holistic diagnosis and treatment, which differs substantially from the principles of orthodox Western-style medicine. Traditional medicine is largely based on the prescription of traditional herbs, known as herbal medicines. The herbs prescribed are believed to work in conjunction to the treatment of diagnosed aliment. In herbal medicines, the herbs are mixed, decocted and generally taken as a traditional tea (Bauer and Tittel, 2004).

Herbal medicines includes herbs, herbal materials, herbal preparations and finished herbal products that contains parts of plants and other plant materials as active ingredients containing a variety of chemical substances that act upon the body (Burkill, 2005).

The safety and efficacy of herbal medicines largely depend on their quality. Requirements and methods for quality control of finished herbal products, particularly for mixture herbal products, are far more complex than for chemical medicines. The quality of finished herbal products is also influenced by the quality of the raw materials used (Bauer and Tittel, 2004).

One of the challenges in analyzing the cause of adverse events reported in connection with use of herbal medicines is the lack of expertise in identifying and testing ingredients and constituents of suspect herbal products at the national pharmacovigilance centres, and/or national quality control laboratories. WHO decided to develop four new documents to provide technical guidance at the key stages where quality control is required in production of herbal medicines: Traditional medicine is the sum total of knowledge, skills and practices based on the theories and experiences indigenous to different cultures that are used to maintain health as well as diagnose improve or treat physical and mental illnesses (Burkill, 2005, WHO, 2011).

The variability of the constituents in herbs or herbal preparations due to genetic, culture and environmental factors has made the use of herbal medicines more challenging than it would necessarily have been. For instance, the availability and quality of the raw materials are frequently problematic. The active principles are diverse and may be unknown, and quality of different batches of preparation may be difficult to control and ascertain, in most countries, herbal products are launched into the market without proper scientific evaluation, and without any mandatory safety and toxicological studies. There is no effective machinery to regulate manufacturing practices and quality standard. Consumers can buy herbal products without a prescription and might not recognize the potential hazards in an inferior product. A well-defined and constant composition of the drug is therefore, one of the most important prerequisites for the production of a quality drug (Byard, 2010).

MATERIALS AND METHODS

Collection of Herbal Products

Total of Ten herbal products were randomly purchased from different traditional stores within Otukpo local Government Areas of Benue State. The samples were thereafter, transported

to the microbiology laboratory, Department of science laboratory technology, Benue State Polytechnic, Ugbokolo, for its antibacterial analysis.

Collection of Isolates

The following clinical isolates: *Escherichia coli, Salmonella typhi, Proteus spp* and *Staphylococcus aureus* were obtained from the Medical Laboratory unit of General Hospital Otukpo and were confirmed by sub culturing on different appropriate selective media and some biochemical tests. The organisms were maintained on agar slop at 4^0C and sub-cultured for 24hours before use.

Susceptibility Analysis

The broth cultures of the test bacterial isolates were incubated at 37^{o}c until it achieves the turbidity of 0.5 McFarland standards (usually 2 to 6 hrs incubation). The isolates were uniformly and aseptically inoculated into a different Mueller-Hinton agar plates by spread plate method using sterile cotton wool. A sterile cotton wool was allowed to soak in the broth culture, squeezed by the side of the bottle before streaking over the sensitivity plates. Then, a hole with a diameter of 8 mm is punched aseptically with a sterile cork borer, and a volume (0.2 ml) of the herbal products was introduced into the well. The plates were then incubated at 37°C for 24 h. The degree of susceptibility of the test isolates to each of the herbal materials was determined by measuring the diameter of the zones of inhibition (Liang *et al*., 2004, Cheesbrough 2006, Adesina *et al*., 2015).

Determination of MIC AND MBC

Dilutions of the inocula were cultured on nutrient agar to verify the absence of contamination and to check the validity of the inoculum. The minimum inhibitory concentrations (MIC), and MBC were performed by a serial dilution technique following the method of Aboaba and Efwape (2011).

The lowest concentrations without visible growth were defined as MICs. The MBCs were determined by serial sub-culturing of 0.5 ml of the tubes that showed inhibition on a solid nutrient Agar medium. The lowest concentration with no visible growth was defined as the MBC (Aboaba and Efwape, 2011; Liang *et al*., 2004).

RESULTS

Table 1: The Product Information of the Herbal Products

Product code	Dosage form	Manufacture dates	Expiry dates	NAFDAC registration number	Therapeutic claims
A	Liquid	03/2016	03/2019	YES	Pile, malaria, typhoid fever, bacterial infection (*Staphylococcus aureus*).
B	Liquid	12/2017	12/2020	YES	*Staphylococcus aureus,*
C	Powder	-	-	-	Body weakness, abdominal pain, nausea.
D	Liquid	03/2017	03/2020	-	Typhoid, malaria, body pain,
E	Liquid	06/2016	06/2019	-	Pile, Bacterial infection, *Staphylococcus aureus*
F	Liquid	03/2017	03/2020	-	Malaria, Syphilis, Typhoid, Constipation.
G	Liquid	08/2016	08/2019	YES	Bacterial infection, pile
H	Powder	-	-	-	Malaria, typhoid fever, pile, *Staphylococcus.*
I	Powder	-	-	-	Abdominal pain, nausea, vomiting and body ache.
J	Liquid	04/2016	04/2019	YES	Treatment for bacterial infection, *Staph. aureus*

Table 2: The Zone of Inhibition of the Samples

Product code	Zone of Inhibition (mm)			
	Staphylococcus aureus	*Salmonella typhi*	*Escherichia coli*	*Proteus spp*
A	8	8	8	8
B	8	8	8	8
C	8	8	8	8
D	8	21	17	8
E	8	8	8	8
F	8	8	32	17
G	8	18	8	8
H	8	8	8	8
I	8	30	36	19
J	8	8	8	8

Table 3: The MIC and MBC of Some of the Herbal Products Used.

Microorganism		Herbal product(MIC (mg / ml)			
		D	F	G	I
E. coli	MIC	50	75	50	25
	MBC	75	100	75	25
S.typhi	MIC	75	25	28	50
	MBC	50	75	70	50
Proteus spp	MIC	57	28	50	75
	MBC	69	30	63	75

DISCUSSION

This study was conducted to validate the antibacterial qualities of some herbal products sold in Otukpo market of Otukpo local government area, Benue State, Nigeria. Ciprofloxacin was used as a negative and positive control. However, for the herbal product the average zone of inhibitions observed against these bacteria ranged from 17 to 36 mm. These values fall within the range of low and moderate sensitivity when compared with control antibiotic.

Samples A,B,D,E,F,G, and J, had manufacture date and expiry date, only A, B, G, and J has NAFDAC registration numbers, showing their reliability, while product C, H, and I have no NAFDAC registration number, no manufacture date and no expiry date (table 1 above). This shows that these products were not regulated by any approved body. Products D, E, F had manufacturing dates, expiry date, but no NAFDAC registration number. Therefore, the products were not regulated by any approved body this result agrees with work of Ameh (2014), that some of the herbal products in Nigeria do not met the regularly bodies specifications.

Table 2 shows the zone of inhibition of all the products used. Product code A, B, C, E, G, H, and J shows resistant to all the bacteria used this could be that the claims were not true. Product code, D, shows resistance to *Staphylococcus aureus*, sensitive to *Salmonella typhi* with, mildly sensitive to *Escherichia coli*, and resistance to *Proteus spp*, this indicates that the product has antibacterial activity against one of the claims made. Product code F shows resistance to *Staphylococcus aureus*, resistant to *Salmonella typhi*, susceptible to *Escherichia coli*, and moderately sensitive to *Proteus spp*, this indicates that the product has antibacterial activity against some of the bacteria used, although this was not included in the claims made. Product G showed mildly sensitive to only *Salmonella typhi*; this product had antibacterial activity for Typhoid which was not part of their claim. Product code I, showed resistance to *Staphylococcus aureus*, resistance to *Salmonella typhi*, sensitive to *Escherichia coli* and mildly sensitive to *Proteus spp*; this indicated that the product had antibacterial activity against some of the test organisms which were not included in the claims. This assessment showed that most of the herbal products do not pass through quality control; most of the claims were estimated. These findings agree with the work of Toge *et al*., (2014) that suggested that product contamination can lead to impaired performance of the product due to disruption of the stability of the formulation and inactivation of the active ingredients.

Table 3: shows the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the effective herbal medicines used. The MIC values ranges from 25to 75, whereas the MBC concentration ranges from 30 to 75. The MBC values were mostly higher than the MIC values obtained. This implies that the effective herbal mixtures were bacteriostatic in lower concentration and bactericidal in higher concentration. The MIC and MBC results agree with the works of Akinyemi *et al.*, (2006) and Adesina *et al.* (2015) that stated that some herbal products, when used traditionally as antibacterial, inhibit bacteria growth without necessarily killing the bacteria. Since most of the traditional preparations lack specific concentrations, this may thus account for the use of large quantity of the herbal products by traditional medicinal practitioners for the treatment of their patients.

Our findings further revealed that the sensitive herbal products were more active against Gram-negative bacteria than Gram-

positive bacteria; which disagrees with most of the claims. Among the tested organisms in this research work *Escherichia coli* showed more sensitivity to the antibacterial action of the herbal products, while *Staphylococcus aureus* showed most resistance to the antibacterial properties of the herbal products used.

CONCLUSION

This study validates the antibacterial claims of herbal products; results showed that not all herbal products sold in Otukpo have antibacterial activities as claimed by the manufacturers. From the findings of this work, it could be concluded that *Escherichia coli* was more sensitive to the herbal products than the other test organisms whereas *Staphylococcus aureus* showed more resistant to most of the herbal products.

RECOMMENDATIONS

- ✓ It is recommended that NAFDAC should always validate the claims of herbal products before registration.
- ✓ It is recommended that the producers of herbal products should properly validate their products before introducing them to the populace.
- ✓ It is recommended that more work be down to validate the antiviral and anti-malarial properties of herbal products.

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