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Evaluation of the Antibacterial Activities of Herbal Medicines Marketed in Osogbo, Osun State, Nigeria

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Abstract: *The search for alternative medicine, prompted by the rise in emergence of antibiotic resistant bacteria led to the manufacture and sales of various local and foreign herbal remedies. Given the variable nature of products of plant origin, it is important to evaluate the effectiveness of commercially available herbal medicines in order to ensure that high quality herbal medicines are developed within or imported into the country. Hence, this study was designed to evaluate and compare the antibacterial properties of selected herbal medicines marketed in Osogbo, Osun state, Nigeria. A total of eight different herbal medicines including four foreign and four local herbal products were used. Untyped clinical isolates of Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa and Staphylococcus aureus obtained from the Medical Microbiology Laboratory of the University of Osun state Teaching Hospital, Osogbo, were used. They were sub-cultured and their identities were confirmed based on their morphological characteristics, Gram's staining and biochemical reactions. The susceptibility of each test bacterium to different herbal drug concentrations was assayed using Agar-well diffusion method. Only 50% of the study's test herbal medicines consisting of two local and two foreign herbal medicines at 100 and 50 mg/mL had significant antibacterial activity against all the test bacteria. Hence, locally produced herbal medicines, can serve as good alternatives to the ones imported, in the treatment of infections, particularly, in the face of the present economic hardship in the country, as they will, in addition to producing good health care outcome, be cost effective.*

Keywords: Antibacterial, Herbal Medicines, Susceptibility, Quality Control.

Introduction

Bacterial infections including respiratory tract infections, skin infections and urinary tract infections pose a significant threat to public health worldwide. They could be caused by a wide range of bacterial pathogens such as *Klebsiella pneumoniae, Escherichia coli, Staphylococcus aureus* and *Pseudomonas aeruginosa* (Spellberg *et al*., 2013). Antibiotics play important roles in the fight against bacterial infections, however, their misuse and prolonged use among people worldwide have resulted in widespread drug resistance which over the years has increased tremendously worldwide (Osman *et al*., 2012) constituting a huge challenge in the treatment of bacterial infections with antibiotics (Foxman, 2014). In the light of this challenge, the need to find new antimicrobial agents became very important. Hence, researchers tirelessly went in search of alternative treatment options (Ventola, 2015) focusing mainly on biologically active plant metabolites such as alkaloids, flavonoids, terpenes, organic acids etc., which have been shown to possess antimicrobial properties (Matasyoh *et al*., 2009). Herbal remedies have been important in addressing the growing challenge of antibiotic resistance (Newman & Cragg, 2012). They have also been shown to be relatively affordable, easily available, producing good treatment outcome with minimal side-effects and reduced toxicity (Lee, *et al*., 2007).

While some consumers in Nigeria are endeared to foreign herbal products than to locally produced ones, because of several factors among which is the marketing strategies and high-quality product packaging employed by well-established foreign companies which increase product appeal, local herbal products also enjoy a good level of patronage as the relatively low cost of locally produced herbal products and the easy accessibility and availability endear these products to their consumers.

Given the variable nature of products of plant origin and the overwhelming increase in the usage of herbal medicines (local and foreign), (Okunlola *et al*., 2007) for treating and preventing infectious diseases, of which many of them are consumed without adequate scientific validation, it is important to evaluate their effectiveness. Several studies have evaluated the effectiveness of various plant extracts on bacterial pathogens but there is paucity of information on the antibacterial potency of both foreign and local herbal medicines that are sold in Osogbo, Nigeria. Hence the need for this study which was designed to evaluate and compare the antibacterial properties of selected liquid local and foreign herbal medicines marketed in Osogbo, Osun state, Nigeria in order to ensure that highly effective herbal medicines are produced locally or imported into the country.

MATERIALS AND METHOD

Washing and Sterilization of Materials

The work bench was sterilized with cotton wool soaked with 95% ethanol. The glass wares were washed with detergent and distilled water and then dried in the hot air oven at $160^0{}_{C.}$

Preparation and Sterilization of Media

The media were prepared according to the manufacturers' instruction.

Sample Collection and Preparation

A total of eight different herbal medicines including four foreign and four local herbal products were purchased from eight different licensed stores of herbal products in Osogbo, Osun state. The samples were kept at room temperature and used within one week of collection. Each of the herbal products was assigned unique identification code. 100 mg/mL stock solution of each powdered herbal medicine was prepared by dissolving 10 grams of the powdered herbal medicine in 100 mL of distilled water. Serial dilutions were then performed from each stock solution to create a range of concentrations including: 50, 25 and 12.5 mg/mL. The contents and therapeutic claims of each test herbal product were noted and are shown in Table 1.

Table 1. The Contents and Therapeutic Claims of Test Herbal Medicines.

Product Code	**Contents**	Therapeutic claim
LhmA	25 different types of roots, herbs, seeds and flowers.	Anti-bacterial and Antifungal.
LhmB	Honey, lime juice, *Zingiber officinale*, and herbal seeds and roots.	Antibacterial, treatment of urinary tract infections and asthmatic cough.
LhmC	Aloe vera plus 31 roots and herbs, fruits and barks.	Antibacterial and antifungal.
LhmD	Natural roots and barks.	Antibacterial, antiviral, purifies blood, detoxifies toxins, builds immune system, stops dizziness and weakness.

FhmA	*Mangifera indica*, *Carica papaya* leaves, Masularia acuminate root, breadfruit bark, citrus lemon leaves, and *Zingiber officinale* root.	Antibacterial and prevention of toothache
FhmB	*Carica papaya* leaves, *Magnifera indica*, bark, *Treculia Africana*, Citrus, *Zingibar officinale* root and *Allium sativum*.	Antibacterial, antirheumatism, reduces sugar and cholesterol with treatment of all kinds of eye infections.
FhmC	*Alium sativum*, Aloe vera bitter, Chick weed, *Preclina nitida*, *Hibiscus sabdrrifa*, Aqua and ethanol.	Antibacterial, antiparasitic, ulcer, constipation, fibroid, internal heat heart burn and diabetes.
FhmD	*Carica papaya*, *Magnifera indica*, Newbouldlia leaves, *Azadricha indica*, *Jaminum officionili*, *Aloe barbedensis and* ginseng.	Antibacterial, antimalarial, antirheumatic, and antiviral.

Test Organisms

Clinical isolates of *Escherichia coli, Klebsiella pneumoniae, Staphylococcus aureus* and *Pseudomonas aeruginosa* obtained from the Medical Microbiology Laboratory of University of Osun state Teaching Hospital, were used for the study. They were sub-cultured and their identities were confirmed based on their morphological characteristics, Gram's staining and biochemical reactions. The cultures were preserved in nutrient agar slants and kept in a refrigerator at 4℃ until required for use.

Antimicrobial Susceptibility Testing

The susceptibility of each test bacterium to each of the different herbal drug concentrations was assayed using Agar-well diffusion method as described in the studies by Ujam *et al*., 2013 and Amankwah *et al*., 2022. Standardized suspensions of each of the test organisms were prepared. About 0.1 mL of each standardized bacterial suspension (0.5 Mcfarland standard) was aseptically transferred into labeled sterile petri dishes after which 20 mL of molten sterile Mueller Hinton agar was poured into the seeded petri dishes. The plates containing bacterial suspension and MH agar were gently swirled and then allowed to solidify after which, holes of depth 3.5 mm were drilled aseptically on the solidified Mueller Hinton agar plates using a 6 mm cork borer. The different concentrations of each herbal medicine (0.1 mL) were dispensed into separate bored holes. Negative control (distilled water) and positive control (Chloramphenicol) were also set up accordingly. All the petri dishes were incubated at 37^0C for 24 hours and were observed for zones of growth inhibition around each well. The resulted zones of inhibition were measured, recorded and interpreted using Kirby-Bauer interpretation chart.

Result

Table 2: Growth of Bacterial Isolates on Different Culture Media

Isolate Code	Nutrient agar	Blood agar	MacConkey agar
BI1	Golden yellow circular, smooth, opaque colonies	Light golden yellow smooth, colonies. Beta haemolysis	No growth
BI2	Greenish blue, smooth, mucoid, opaque colonies	Greyish white, smooth, mucoid, opaque colonies. Beta haemolysis	Colourless, smooth, mucoid, transparent colonies.
BI3	Large, greyish white smooth, mucoid, translucent colonies	Greyish white, mucoid, translucent colonies Beta haemolysis	Pink, smooth, mucoid, opaque colonies
BI4	Greyish white, mucoid, translucent colonies	Greyish white, mucoid, translucent colonies Gamma haemolysis	Large, pink, mucoid, opaque colonies

KEY; BI1= Bacterial isolate 1, BI2= Bacterial isolate 2

Table 3: Characterization and Identification of the Bacterial Isolates

Bacterial Isolates	Microscopy	Gram Staining	Catalase	Indole	Citrate	Methyl Red	Oxidase	Lactose Fermentation	Possible bacteria
BI1	Cocci arranged in clusters	+ve	+ve	+ve	-ve	+ve	-ve	+ve	*Staphylococcus aureus*
BI2	Rod shape	-ve	+ve	-ve	ND	-ve	+ve	-ve	*Pseudomonas aeruginosa*
BI3	Short rods	-ve	+ve	+ve	ND	+ve	-ve	+ve	*Escherichia coli*
BI4	Rod/ cocci	-ve	+ve	-ve	+ve	-ve	-ve	+ve	*Klebsiella pneumoniae*

KEY; BI1= Bacterial isolate 1, BI2= Bacterial isolate 2, BI3=Bacterial isolate 3, BI4=Bacterial isolate 4, ND = Not Detected, +ve = Positive, -ve = Negative

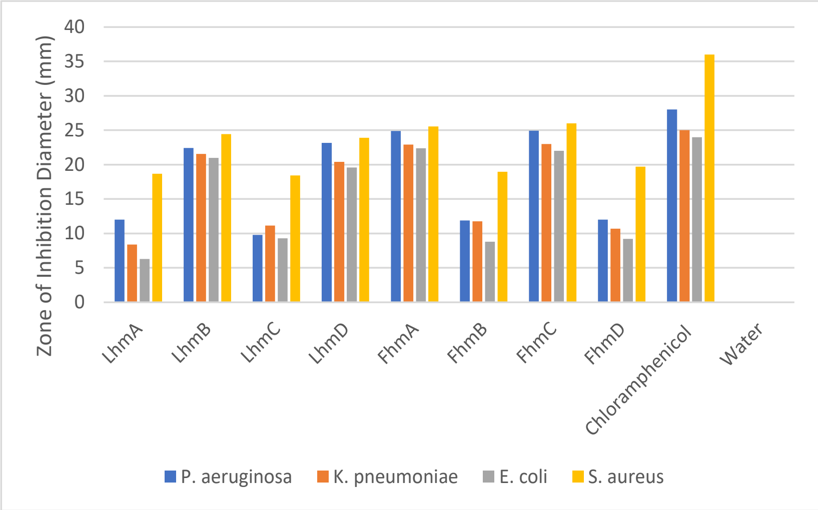

Figure 1: Comparative Antibacterial Activity of Local and Foreign Herbal Medicines Against Test Bacteria at 100 mg/ml

Key:
LhmA- Local herbal medicine A
LhmB- Local herbal medicine B
LhmC- Local herbal medicine C
LhmD- Local herbal medicine D
FhmA- Foreign herbal medicine A
FhmB- Foreign herbal medicine B
FhmC- Foreign herbal medicine C
FhmB- Foreignl herbal medicine D

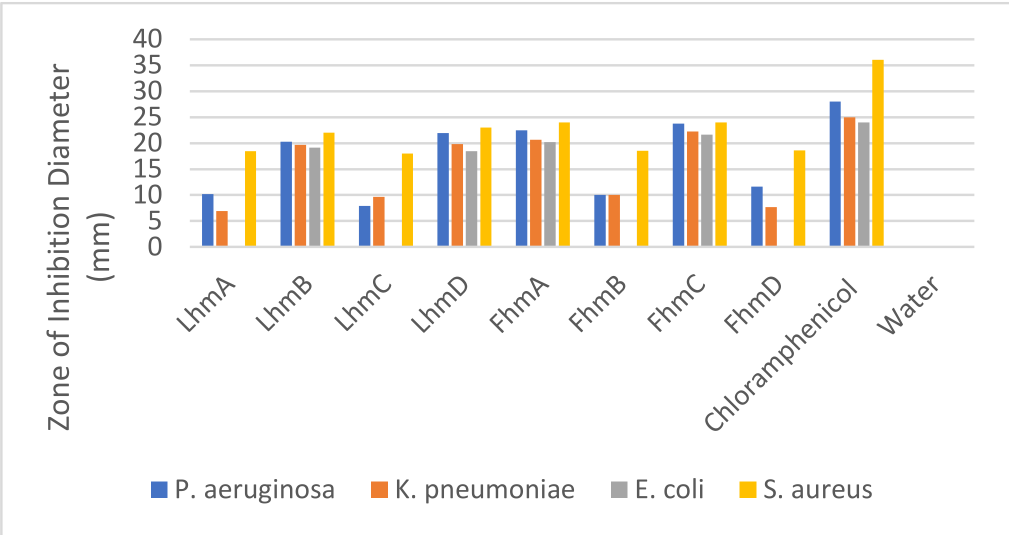

Figure 2: Comparative Antibacterial Activity of Local and Foreign Herbal Medicines Against Test Bacteria at 50 mg/ml

Key:
LhmA- Local herbal medicine A
LhmB- Local herbal medicine B
LhmC- Local herbal medicine C
LhmD- Local herbal medicine D
FhmA- Foreign herbal medicine A
FhmB- Foreign herbal medicine B
FhmC- Foreign herbal medicine C
FhmB- Foreignl herbal medicine D

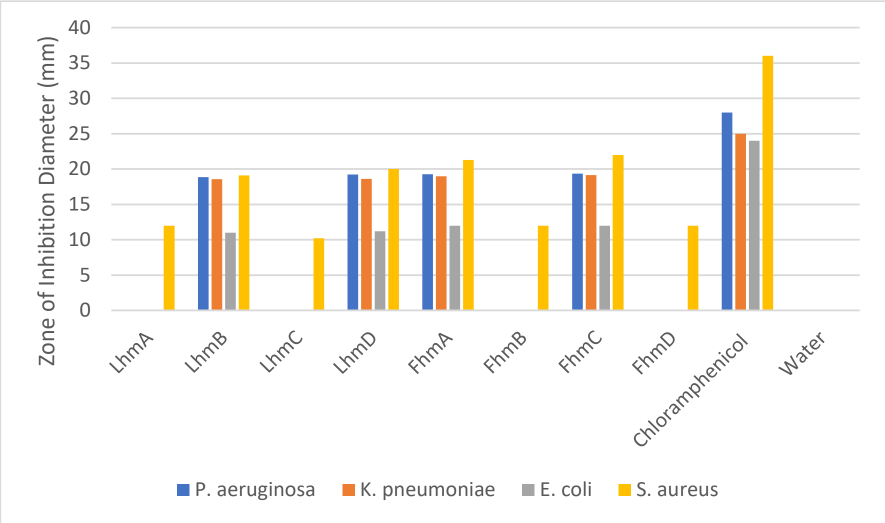

Figure 3: Comparative Antibacterial Activity of Local and Foreign Herbal Medicines Against Test Bacteria at 25mg/ml

Key:

LhmA- Local herbal medicine A LhmB- Local herbal medicine B LhmC- Local herbal medicine C
LhmD- Local herbal medicine D FhmA- Foreign herbal medicine A FhmB- Foreign herbal medicine B
FhmC- Foreign herbal medicine C FhmB- Foreignl herbal medicine D

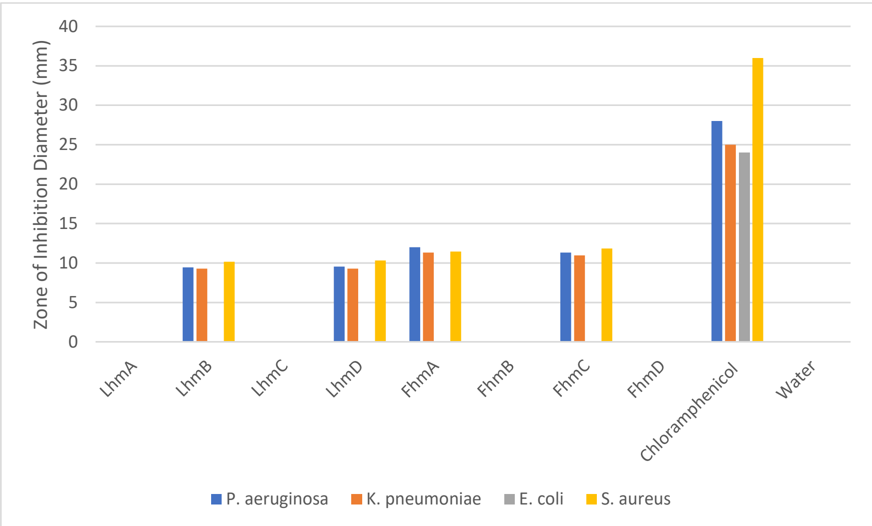

Figure 4: Comparative Antibacterial Activity of Local and Foreign Herbal Medicines Against Test Bacteria at 12.5mg/ml

Key:

LhmA- Local herbal medicine A LhmB- Local herbal medicine B LhmC- Local herbal medicine C
LhmD- Local herbal medicine D FhmA- Foreign herbal medicine A FhmB- Foreign herbal medicine B
FhmC- Foreign herbal medicine C FhmB- Foreignl herbal medicine D

The different herbal products demonstrated different antibacterial activities at different concentrations, with zones of inhibition decreasing as the concentration decreased.

At 100 mg/mL, the zones of inhibition produced by local herbal medicines A, B, C and D around *Pseudomonas aeruginosa* were 12 mm, 22.43 mm, 9.8 mm and 23.16 mm respectively. Zones of inhibition including 8.4 mm, 21.56 mm, 11.14 mm and 20.42 mm were also recorded with the use of the same local herbal medicines A, B, C and D around *K. pneumoniae.* At the same concentration of 100 mg/mL, foreign herbal medicines A, B, C and D produced zones of inhibition measuring 24.89 mm, 11.9 mm, 24.92 mm and 12 mm around *Pseudomonas aeruginosa*. *K. pneumoniae*, on the other hand, showed zones of inhibition measuring 22.93 mm, 11.75 mm, 23 mm and 10.7 mm with the use of the same foreign herbal medicines A, B, C and D at the afore mentioned concentration. For local herbal medicines A, B, C and D, the zones, of inhibition around *E. coli* measured 6.3 mm, 21 mm, 9.3 mm and 19.59 mm respectively while for foreign herbal medicines A, B, C and D, it measured 22.38 mm, 8.8 mm, 22 mm and 9.2 mm respectively at 100 mg/mL. *S. aureus* showed zones, of inhibition that measured 18.68 mm, 24,45 mm, 18,43 mm and 23.89 mm with the use of local herbal medicines A, B, C and D respectively and zones of inhibition that measured 25.54 mm, 18.96 mm, 26 mm and 19.69 mm when foreign herbal medicines A, B, C and D were used respectively at 100 mg/mL (Figure 1).

At 50 mg/mL, *Pseudomonas aeruginosa* showed zones of inhibition that measured 10.15 mm, 20.26 mm, 7.9 mm and 21.96 mm using local herbal medicines A, B, C and D respectively and zones of inhibition that measured 22.44 mm, 10 mm, 23.76 mm and 11.62 mm using foreign herbal medicines A, B, C and D respectively. At the same concentration, *K. pneumoniae* showed zones of inhibition that measured 6.9, 19.63, 9.69 and 19.79 mm with the use of local herbal medicines A, B, C and D respectively and zones of inhibition that measured 20.66, 10, 22.27 and 7.7 mm when foreign herbal medicines A, B, C and D were used respectively. For local herbal medicines A, B, C and D, at 50 mg/mL, the zones, of inhibition around *E. coli* measured 0 mm, 19.15, 0 mm and 18.46 mm respectively while for foreign herbal medicines A, B, C and D, it measured 20.17 mm, 0 mm, 21.65 and 0 mm respectively. *S. aureus* showed zones, of inhibition that measured 18.45 mm, 22 mm, 18 mm and 23 mm with the use of local herbal medicines A, B, C and D respectively and zones of inhibition that measured 23.95 mm, 18,54 mm, 24 mm and 18.63 mm respectively when foreign herbal medicines A, B, C and D were used respectively (Figure 2).

At 25 mg/mL, no zone of inhibition was recorded for *P. aeruginosa, K. pneumoniae* and *E. coli* using local herbal medicines A and C and foreign herbal medicines B and D respectively. However, zones of inhibition measuring 18.84 mm and 19.23 mm for local herbal medicines B and D and zones measuring 19.26 mm and 19.36 mm for foreign herbal medicines A and C were recorded for *P. aeruginosa.* Zones of inhibition measuring 18.57 mm and 18.63 mm for local herbal medicines B and D and zones measuring 18.97 mm and 19.14 mm for foreign herbal medicines A and C were recorded for *K. pneumoniae.* Zones of inhibition measuring 11 mm and 11.21 mm for local herbal medicines B and D and zones measuring 12 mm each for foreign herbal medicines A and C were recorded for *E. coli. S. aureus* however showed zones of inhibition that measured 11.98 mm, 19.1 mm, 10.23 mm and 20 mm with the use of local herbal medicines A, B, C and D respectively and zones of inhibition that measured 21.3 mm, 12 mm, 22 mm and 12 mm respectively when foreign herbal medicines A, B, C and D were used respectively (Figure 3).

At the lowest concentration of 12.5 mg/mL, no zone of inhibition was recorded for *P. aeruginosa*, *K. pneumoniae* and *S. aureus* using local herbal medicines A and C and foreign herbal medicines B and D respectively. *P. aeruginosa* however, showed zones of inhibition that measured 9.45 mm and 9.56 mm for local herbal medicines B and D and zones of inhibition that measured 12 mm and 11.34 mm for foreign herbal medicines A and C. At this same concentration, *E. coli*, showed no zone of inhibition with the use of any of the test samples. *S. aureus however*, showed zones, of inhibition that measured 10.17 mm and 10.34 mm for local herbal medicines B and D respectively and zones of inhibition that measured 11.46 mm and 11.84 mm with the use of foreign herbal medicines A and C respectively (Figure 4).

Discussion

All the eight brands of herbal medicines used in this study had antibacterial claims. Four out of the eight were made in Nigeria while the remaining four were imported from outside the country. All the herbal products had NAFDAC registration number. The contents of the test herbal medicines were provided on leaflets packaged with the preparations as shown on Table 1., unlike in the study by Temu-Justin, *et al*., 2011 and Onyewenjo, *et al*., 2022 which revealed that the majority of herbal medicinal products sold in South-Western Nigeria were not registered by NAFDAC and did not have their content stated. All of the herbal medications sampled in this study, had their expiry and manufacturing dates stated just like in the study by Akujobi, *et al*., 2006. This study showed *Escherichia coli* to be the least sensitive to the antibacterial activities of all the test herbal medicines (Figures 1-4). The herbal medicines in this study also showed pronounced activity on *S. aureus* (Figures 1-4). This result is contrary to that obtained in the study by Onyewenjo, *et al*., 2022 in which *Salmonella typhi* was resistant to all the test samples and *E. coli* showed susceptibility to the herbal remedies. The response by *Escherichia coli* in this study could be attributed to; its possession of many efflux pumps responsible for pumping out inhibitory agents and reducing their concentrations (Mario, 2020) and also to the nature of its cell envelope which is known to inhibit the passage of many antimicrobial agents (Ahmadu *et al*., 2006). The result of this work again deviates from the result of the study by Czech, *et al*., 2001 that demonstrated that herbal preparations showed good susceptibility to tested organisms even in the least concentrations of 5 μl/mL as the least concentration of 12.5 mg/mL in this study was not able to inhibit the growth of any of the test organisms (Figures 4).

The table revealed that four (4) out of the eight (8) herbal medicines inhibited the growth of all the test bacteria at 100 and 50 mg/mL concentrations. Local herbal medicines B and D and foreign herbal medicines A and C had the highest inhibitory activity at 100 and 50 mg/mL concentrations as all the test bacteria showed susceptibility to these herbal test samples (Figures 1 and 2). All the test bacteria except *S. aureus* showed resistance to the local herbal medicines A and C and foreign herbal medicine B and D at the afore mentioned concentrations (Figures 1 and 2). These herbal products (local herbal medicines A and C and foreign herbal medicine B and D) showed the lowest inhibitory activity (Figures 1 and 2).

All the test bacteria at 25 mg/mL showed resistance to the local herbal medicines A and C and the foreign herbal medicine B and D. Conversely, all the test bacteria except *E. coli* displayed susceptibility to the remaining four herbal medicines including the local herbal medicines B and D and the foreign herbal medicines A and *C* as inhibition zone diameter (IZD) ≥18 mm. Thus, the local herbal medicines B and D and the foreign herbal medicines A and C showed significant antibacterial activity at 25 mg/mL concentration against all the test bacteria except against *E. coli* (Figure 3). At the lowest concentration of 12.5 mg/mL however, none of the test bacteria was susceptible to any of the test samples in this study as inhibition zone diameter (IZD) ≤12 (Figure 4).

In summary, greater antibacterial activities were observed at higher concentrations of 100 and 50 mg/mL concentrations (Figures 1 and 2). Only two out of the four local herbal medicines and two out of the four herbal medicines of foreign origin displayed significant antibacterial activities against most of the test bacteria in this study (Figures 1, 2 and 3). Despite the fact that all the products had antibacterial claims, only 4 (50%) of the herbal products, (LhmB, LhmD, FhmA and FhmC) displayed significant antibacterial potency. This was similar to the result obtained in the study by Ujam *et al*., 2013 in which (50%) of the herbal products showed antibacterial activities. The poor antimicrobial activity shown by the herbal products against the test organisms in this study could be attributed to insufficient active ingredients in the products which could be due to errors in the extraction process or the presence of microbial contaminants which were however not assessed in this study but which are known to cause degradation of active ingredients contained in herbal preparations and also shown to be able to enter via the production environment or improper handling of medicinal plants (Idu *et al*., 2010; Oleghe *et al*., 2011).

Conclusion and Recommendations

Local herbal medicines B and D and foreign herbal medicines A and C were the most effective in antibacterial potency while local herbal medicines A and C and foreign herbal medicine B and D were the least effective in antibacterial activities. Since some local herbal products can also be as effective as foreign herbal products as shown in this study, the use of high-quality, locally produced herbal medicines, can serve as good alternatives to the ones imported from foreign countries, in the treatment of infections particularly in the face of the present economic hardship in Nigeria as they will in addition to producing good health care outcome, be cost effective. However, stricter quality control measures should be established and implemented in herbal medicine production by regulatory bodies to ensure the safety, consistency and potency of both local and foreign herbal products. They should also develop and enhance post-market surveillance programs to continuously monitor the safety and efficacy of herbal products in the market as this can help identify any emerging issues or changes in efficacy over time. Finally, the Nigerian Government, should fund and support research into the development of more effective local herbal products as this will go a long way in producing and making numerous effective, high-quality and safe local herbal medicines available at more affordable prices.

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