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Microbiological Analysis, Quality and Safety Evaluation of Commercially Sold Medicinal Herbal Cocktails from Local Herb Seller within Akoko South West, Ondo State, Nigeria

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

The use of herbal plants has been growing rapidly worldwide; it has been widely and extensively used locally as medicinal products in the treatment of different diseases. The extensive use of herbal medicinal products in the treatment and management of disease made it imperative to investigate the microbial analysis of locally prepared herbal cocktails sold in Akoko South West. Nigeria Different samples of local herbs were randomly collected from different locations into sterile polythene and transported to the microbiology laboratory for further microbial analysis. The samples were analyzed using pour plate techniques. The bacteria isolates were characterized and identified based on their colonial, morphological characteristics, and biochemical tests according to Bergey's Manual of Determinative Bacteriology. Antibiotic susceptibility test of the isolates was

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carried out using the disc agar diffusion method. The killing rate and growth dynamics of the bacterial isolates were determined using a Ultra-violet spectrophotometer. Twelve species of the isolates were recovered. *Staphylococcus aureus* and *Clostridium sporogenes* showed a high susceptibility rate to Ciprofloxacin, levofloxacin, Gentamicin, ampiclox, rifampicin, and Amoxillin while they were both resistant to Streptomycin, Norfloxacin, chloramphenicol, and Erythromycin. The Gram-positive isolates were 100% resistant to Norfloxacin, chloramphenicol, Erythromycin and 100% sensitive to Ciprofloxacin. UV-VIS spectrophotometer was used to measure the material of absorbance and quantitative analysis at the visible or ultraviolet light (200 ~ 760nm). Ultra-violet (UV) spectrophotometer was used to determine the growth dynamics and killing kinetics of isolated organisms, and to predict the wavelength of killing ratio of organisms isolated from the herbal cocktail. The addition of antibiotics to the isolated organisms at the 84th-hour speed up the death rate of the isolates from commercially sold herbal cocktail between 450-480nm wavelengths. Water used for production of herbal cocktail needed prolong hours of exposure to Ultraviolet rays/ light is necessary, to reduce microbial load drastically, with a great effect on the quality and safety of commercially sold herbal cocktail. There is a need for constant monitoring and quality control of herbal medicinal products being manufactured, sold, and used in Nigeria so as to reduce and or eradicate the effect of the organisms on human health.

Keywords: Herbal; evaluation; microbial qualities; herbal cocktail; microbiological analysis.

1. INTRODUCTION

The beneficial medicinal effects of plant materials typically result from the combinations of secondary products present in the plants. Compounds extracted from different parts of the plants have been used to cure diarrhea, dysentery, cough, cold, cholera, fever, bronchitis, etc. According to [1] 35,000 to 70,000 plant species have been used in folk medicine worldwide. The use of medicinal plants as a drug is an alternative method for the management of pathogenic microbes like bacteria, fungi, and viruses and is eco-friendly. However, there is a widespread perception that herbs are inherently safe, incidences of intoxication following the use of these herbs have been reported in different parts of the world [2].

Herbs and spices are common parts of the food for flavor, color, aroma, taste, and also to enhance shelf life. Some of the common herbs viz. Arjuna, Ashwagandha, Puthkanda, and Shalampanja were tested for antimicrobial activity. The chemical and microbial quality of herbal plants and their formulations depend on various factors including geography, climatic conditions, water, soil and air pollution, growth, transport and storage conditions, and many other environmental factors [3].

In Nigeria, the use of local herbs in the form of concoction and decoction treatments for various infections is a common practice, with approximately 75% of the rural population relying on this practice [4]. *Uvaria chamas* has been used in the South-East of Nigeria to treat feverish condition, malaria, and body pains [5]. Other herbs such as the *Azadirachta indica, Aleovera*spp.*, Psiduim guava, Carica papaya,* and *Cymbopogen citrates* are used for various ailments such as malaria fever, headache, body pains, skin infections, stomach, diarrhea and simply by use of different methods of applications. Moreover, it serves as a cheaper means of treatment for low-income earners who cannot afford the cost of modern therapies used for the treatment of typhoid fever. The widespread use of herbal concoctions or medicines calls for the assurance of sustainable availability of quality and safe preparations of these herbs in order to guarantee their continued access especially for rural or low-income communities, without compromising patient quality and safety [6]. Many microbial contaminants can alter the physicochemical features which can then lead to mischievous changes to the quality of herbal preparations [7].The rapidly expanding markets for herbal preparations use clearly necessitate assessing issues concerning the quality and safety of these products for end-users [8].

Herbal plants are a natural source of compounds that can be used against many diseases today. Informal street merchants and traditional health practitioners primarily offer consumers semi-processed herbal preparations that are commonly prepared in small batches. In the preparation of the herbal concoctions, fresh or dry plant material can be used. The plant material can either be macerated in water for several days or generally boiled in hot water [9]. Moreover, the increased cost of new and more effective antimicrobial remedies together with their side effects and lack of health care facilities in some rural areas [10], makes the search for safer, more effective, and affordable alternative remedies imperatives.In South Africa, herbal products that are sold by informal traders are usually claimed to be immune and energy boosters, blood cleansers, detoxifiers, and aphrodisiacs [11].There also seemed to be a proportional high demand for plant-based medicines, in that the estimated annual market value of phytomedicines stood at 75 to 150 million USD [12,13].

The broad use of traditional herbal remedies has encouraged manufacturers, private traders, and street merchants to capitalize on this upsurge by increasing the availability of herbal remedies to those who desire them [14]. The signs of urbanization are witnessed by the increase in herbal shops, informal street traders, and the wide distribution of herbal remedies in pharmacies and supermarkets [15].

For the herbal cocktails to be reputable, maintain quality, reliability, and marketable, they must meet quality health standards. However, investigating herbal concoction quality and safety is accompanied by the challenge that some herbalists, traditional healers, and/or traders are reluctant to divulge the ingredients and formulae of some of their products. Hence this study.

2. METHODS

2.1 Sample Collection of Commercially Sold Herbal Cocktail

Different samples of local herbs were randomly collected from different locations namely; Epo Mango and Ogi were used for typhoid fever treatment, Opaeyin herb made of epomogani, Epoayi and Egboati herb for Jedi, Omi osan, ewe iba, typhoid mixture used for malaria treatment, Agunmu gbogbonisi mixed with either Jedi liquid herb or typhoid. Afato herb made by the mixture of Igi mango was pounded and squeezed, then soaked with Ogi water to cure back pain was also collected All samples were collected into sterile polythene and transported to a microbiology laboratory for further microbial analysis [16].

2.2 Microbial Analysis of a Commercially Sold Herbal Cocktail

The samples were analyzed using pour plate techniques to obtain the total bacterial count (TBC) in colony-forming units per ml (CFU/ml). Briefly, 1 ml of each of the local herbal concoctions is dispensed into a test tube containing 9 ml of sterile distilled water and shaken to obtain 10^{-1}diluent. The samples were further diluted to 10^{-5}. 1 ml of dilutions $10^{-3}$to 10^{-5} of the herbal concoction was aliquoted into a sterile petri dish and was plated under

Image 1. Commercially sold medicinal herbal cocktail local herb seller within Akoko South West, Ondo State, Nigeria

the aseptic condition to prevent contamination and incubated at 37^{o}Cfor 24hrs. After 24hrs, the cultural and morphological characteristics were examined and recorded (17). Colonies observed were picked and subculture severally to obtain a pure culture of the isolates and were stored at 4^{o}C for further use.

2.3 Characterization and Identification of Bacteria Isolates from Commercially Sold Herbal Cocktail

The bacteria isolates were characterized and identified based on their colonial, morphological characteristics and biochemical tests such as methyl red, Vogues-Proskauer, Citrate, Urease, Indole, Motility, Catalase, Oxidase, and Sugar fermentation tests according to Bergey's Manual of Determinative Bacteriology [18,19].

2.4 Antibiotics Susceptibility Testing of a Commercially Sold Herbal Cocktail

Antibiotics susceptibility test of the isolates was carried out using disc agar diffusion method. Antibiotics such as Tarvid (OFX-10mcg), Reflacine (PEF-10mcg), Ciprofloxacin (CPX-10mcg), Augmentin (AU-30mcg), Gentamicin (CN-10mcg), Streptomycin (S-30mcg), Cepofex (CEP-10mcg), Nalidixic acid (NA-30mcg), septrin (SXT-30mcg), ampicillin (PN-30mcg) was used againstGram-negative isolates while ciprofloxacin (CPX-10mcg), Norfloxacin (NB-10mg), gentamicin (CN-10mcg), Amoxicillin (AML-20mcg), streptomycin (S-30mcg), rifampicin (RD-20mcg), Erythromycin (E-30mcg), chloramphenicol (CH-30mcg), ampiclox (APX-20mcg) and levofloxacin (LEV-20mcg for Gram positive bacteria [20].

2.5 Killing Rate and Growth Dynamics of Bacterial Isolates from Commercially Sold Herbal Cocktail

An 18-24hours old culture was used to determine the growth dynamic and killing rate of the bacteria isolates using an ultraviolet spectrophotometer. Briefly, the sterile nutrient broth was inoculated with a loopful of 18-24hours old isolate. Five of the test tube was used for growth dynamics and labeled as sample A and one serves as the control. Another five test tubes were used for killing rate as sample B, the killing rate and dynamic growth were observed using an ultra-violet spectrophotometer at a wavelength of 480nm between 0hours to 84 hours, and readings were taken and recorded [21].

3. RESULTS

Table 1 depicted the sample types and locations of a commercially sold herbal cocktail. The samples were obtained from six different locations namely; Ikare, Apex junction, Okusa market, Ibaka market, Okota market, and Chicken republic junction in Akungba Akoko. The highest colony count was obtained from Opaeyin local herb concoction with 360x10^{-5} CFU/ml as depicted in Fig. 1. The total bacteria counts ranged between 20 and 280x10^{-5}. The highest number of colonies was obtained in Opaeyin herbal cocktail with 360 counts followed by Jedi (280) at dilution 10^{-3}and Malaria herbal cocktail (Iba) (172) at dilution 10^{-5}while the lowest was obtained in Afato with 23 colonies at dilution 10^{-5}.

Table 2 showed the morphological characteristics of isolates showing their size, colour, texture, opacity edge, and shape. Jedi herbal cocktail, dilution factor 10^{-3} and 10^{-5} has a creamy colour, rough texture, irregular shape, rough edge, opaque and dilution factor10^{-5} has a small size while Jedi herbal cocktail dilution factor 10^{-3} has a medium size. Opa-eyin herbal cocktail dilution factor 10^{-3} and 10^{-5} has a medium size, rough texture opaque, rough edges, and irregular shape. Dilution factor 10^{-3} has a cream colour white. Dilution factor 10^{-5} has a whitish colour. Typhoid cocktail dilution factor 10^{-3} and 10^{-5} has small size, whitish colour, opaque, rough edges, and irregular shape. Iba herbal cocktail dilution factor 10^{-3} and 10^{-5} has a rough texture, opaque, rough edges, and irregular shape. Dilution 10^{-3} has a medium size and whitish colour dilution factor 10^{-5} has a small size and cream colour. Agunmu herbal cocktail dilution factors 10^{-3} and 10^{-5} has rough textures opaque, rough edges, and irregular shape. Dilution factor 10^{-3} has a medium size and cream colour dilution factor 10^{-5} has size and whitish colour. Afato herbal cocktail dilution factor 10^{-3} and 10^{-5} has small size, cream colour, smooth edges, opaque, smooth texture, and regular shape.

Table 3 showed Gram staining and microscopic examination of the isolates from a commercially sold herbal cocktails. it was observed in the table that S1 Jedi herbal cocktail dilution factor (10^{-3}), Jedi herbal cocktail (10^{-5}),Opa-eyin herbal cocktail dilution factor (10^{-3}), Typhoid herbal cocktail dilution factor(10^{-5}),Malaria herbal cocktail dilution factor (10$^{-3)}$,(10^{-5}), Agunmu herbal cocktail dilution factor (10^{-5}), Afato herbal cocktail dilution factor (10^{-3}),(10^{-5})were positive to Gram staining. S_1 Jedi herbal cocktail dilution factor (10^{-3}), S_2 Opa-eyin herbal cocktail dilution factor (10^{-3}), s4 Malaria herbal cocktail dilution factor (10^{-3}),(10^{-5}), Afato herbal cocktail dilution factor (10^{-3}), (10^{-5}) has small rod.. S_2 Opa-eyin herbal cocktail dilution factor(10^{-5}),S_3-Typhoid herbal cocktail dilution factor (10^{-5}), S5 Agunmu herbal cocktail dilution factor (10^{-3}) were negative for Gram staining.

The antibiotic susceptibility profile of the bacteria isolated from commercially sold herbal cocktails was depicted in Fig. 2. *Staphylococcus aureus* and *Clostridium sporogenes* showed a high susceptibility rate to Ciprofloxacin (CPX), levofloxacin (LEV), Gentamicin (CN), ampiclox (APX), rifampicin (RD) and Amoxicillin (AMX) while they were both resistance to Streptomycin (S), Norfloxacin (NB), chloramphenicol (CH) and Erythromycin (E). All the Gram-positive isolates were 100% resistant to Norfloxacin (NB), chloramphenicol (CH), Erythromycin € and 100% sensitive to Ciprofloxacin(CPX). *Micrococcus luteus* was resistant to six of the antibiotics namely Norfloxacin (NB), chloramphenicol (CH), Erythromycin (E), levofloxacin (LEV), ampiclox (APX), Amoxicillin (AMX) and *Mycobacterium lactum* showed resistance to Norfloxacin (NB), chloramphenicol (CH), Erythromycin (E), levofloxacin (LEV), CN, ampiclox (APX), RD. 62.5% of the isolates were resistant to ampiclox (APX) and Amoxicillin (AMX). *Salmonella paratyphi,* showed resistance to PN, Reflacine (CEF), NA, AU, SXT while it was sensitive to S, OFX, PEF, CPX, CN as reported in Fig. 2. *Citrobacter freundii*, *Chromobacterum violaceum,* and *Cellulomonas biazotea* showed 100% to PN, CEF, NA, and SXT while all are sensitive to OFX and PEF at 100%.

Table 1. Sample type, location and commercially sold herbal cocktail

Sample Type	Location	Source/Herbal preparation
Typhoid	Apex junction Akungba	Epo mango+ pap water
Opa-eyin	Okusa market	Epomogani
Jedi	Ibaka market	Epoayi + Egboati
Iba(Malaria)	Okoja Market Ikare	Omi osan
Typhoid	Ikare junction	Ewe iba
Agunmu	Ikare	Gbogbonise
Afato	Chicken rep. junction	Pounded Igi mango and squeezed then soaked with pap water
Typhoid+Afato	Ikare junction	Combination of both ingredient
Jedijedi + Opa-eyin	Apex junction	Combination of both liquid mixture

Table 2. Macroscopic morphological characteristics of isolates from commercially sold herbal cocktail

Samples From Commercially Sold Herbal Cocktail	Size	Colour	Texture	Opacity	Edge	Shape
Jedi(J^1) 10^{-5}	Small	Cream	Rough	Opaque	Rough	Irregular
Jedi (J^2) 10^{-3}	Medium	Cream	Rough	Opaque	Rough	Irregular
Opa Eyin(O^1) 10^{-5}	Medium	Whitish	Rough	Opaque	Rough	Irregular
Opa Eyin(O^2) 10^{-3}	Medium	Cream	Rough	Opaque	Rough	Irregular
Typhoid Asapo(Ta^1) 10^{-5}	Small	Whitish	Rough	Opaque	Rough	Irregular
Typhoid Asapo (Ta^2) 10^{-3}	Small	Whitish	Rough	Opaque	Rough	Irregular
Malaria Asapo (Iba)(Ma^1) 10^{-3}	Medium	Whitish	Rough	Opaque	Rough	Irregular
Malaria Asapo (Iba) (Ma^2) 10^{-5}	Small	Cream	Rough	Opaque	Rough	Irregular
Agunmu Asapo (Aga^1) 10^{-3}	Medium	Cream	Rough	Opaque	Rough	Irregular
Agunmu Asapo (Aga^2) 10^{-5}	Small	Whitish	Rough	Opaque	Rough	Irregular
Afato Asapo (Afa^1) 10^{-3}	Small	Cream	Smooth	Opaque	Smooth	Regular
Afato Asapo (Afa^2) 10^{-5}	small	Cream	Smooth	Opaque	Smooth	Regular

Table 3. Gram Staining and microscopic examination of isolates from commercially sold herbal cocktail

Samples From Commercially Sold Herbal Cocktail	Gram staining	Shape
Jedi(J^1) 10^{-5}	+ve	Small rod
Jedi (J^2) 10^{-3}	+ve	Cocci
Opa Eyin(O^1) 10^{-5}	+ve	Small rod
Opa Eyin((O^2) 10^{-3}	-ve	Rod
Typhoid Asapo(Ta^1) 10^{-5}	-ve	Rod
Typhoid Asapo (Ta^2) 10^{-3}	+ve	Cocci
Malaria Asapo (Iba)(Ma^1) 10^{-3}	+ve	Small rod
Malaria Asapo (Iba) (Ma^2) 10^{-5}	+ve	Small rod
Agunmu Asapo (Aga^1) 10^{-3}	-ve	Rod
Agunmu Asapo (Aga^2) 10^{-5}	+ve	Cocci
Afato Asapo (Afa^1) 10^{-3}	+ve	Small rod
Afato Asapo (Afa^2) 10^{-5}	+ve	Small rod

Table 4 shows the biochemical test of isolates from commercially sold herbal cocktails.S_1 Jedi 10^{-3} and 10^{-5} herbal cocktail. Indicate was negative to motility test, Indole, Urease and Oxidase, Triple Sugar fermentation {TSI} lactose, Sucrose, and Dextrose. Catalase, H_2S with gas production.S_2 Opa-eyin 10^{-3}, S_3 Typhoid 10^{-5}, S_4 Malaria 10^{-5}, and S_5 Agunmu10^{-5} herbal cocktail, were negative for motility, Indole, Sucrose, Urease test. S_3 Typhoid 10^{-3} was positive to Motility, TSI, urease, gas production, and moderate sugar utilization for Catalase, reacting positively to H_2S and Oxidase. S_4 Malaria 10^{-3} herbal cocktails react positively to Motility test, Indole, Sucrose, Dextrose, Moderate Sugar utilization for Catalase, positive to H_2S, Gas production, and Oxidase while it reacts negatively to Lactose and Urease .S_5 Agunmu 10^{-3} herbal cocktail reacts positively to Motility, Sucrose, Dextrose, Catalase, H_2S, Oxidase test, Indole, Lactose, gas production, and Oxidase.S_6 Afato 10^{-3}, 10^{-5} herbal cocktail were positive to motility, TSI, Catalase, Gas production, and Oxidase test while S_6 10^{-3} reacts negatively to Indole, Urease test, gas production H_2S, and S_6 10^{-5} react negatively to Sucrose, Lactose, Urease, and H_2S.

Twelve species of the isolates and their identity was revealed to be *Streptococcus lactis, Staphylococcus aureus, Citrobacter freundii, Micrococcus luteus, Salmonella paratyphi, Bacillus subtilis, Lactobacillus casei, Sarcina flava, Mycobacterium lactum, Chromobacterum violaceum, Cellulomonas biazotea,* and *Clostridium sporogenes h*as shown in Table 5.

Table 4. Biochemical Tests of Isolates from commercially sold Herbal Cocktail

Samples From Commercially Sold Herbal Cocktail	Motility	Indole	Sucrose	Lactose	Dextrose	Catalase	Urease	H_2S	Gas production	Oxidase
Jedi(J^1) 10^{-5}	-	-	+	+	+	++	-	+	+	-
Jedi (J^2) 10^{-3}	-	-	+	+	+	++	-	+	+	-
Opa Eyin(O^1) 10^{-5}	-	-	+	-	+	++	-	+	-	-
Opa Eyin (O^2) 10^{-3}	+	+	+	+	+	++	-	+	-	-
Typhoid Asapo(Ta^1) 10^{-5}	+	-	-	-	-	++	-	+	-	+
Typhoid Asapo (Ta^2) 10^{-3}	-	-	+	+	+	++	-	+	+	+
Malaria Asapo (Iba)(Ma^1) 10^{-3}	+	+	+	-	+	++	-	+	+	+
Malaria Asapo (Iba) (Ma^2) 10^{-5}	-	-	-	-	+	++	-	+	-	+
Agunmu Asapo (Aga^1) 10^{-3}	+	-	+	-	+	++	-	+	-	-
Agunmu Asapo (Aga^2) 10^{-5}	-	-	+	+	+	++	-	+	-	-
Afato Asapo (Afa^1) 10^{-3}	+	-	+	+	+	+++	-	-	+	+
Afato Asapo (Afa^2) 10^{-5}	+	+	-	-	+	++	-	-	+	+

KEY: + = positive, - = negative, ++ = moderate sugar utilization

Table 5. Probable identity of bacteria isolates from commercially sold herbal cocktail

Samples From Commercially Sold Herbal Cocktail	Probable Identity
Jedi(J^1) 10^{-5}	*Staphylococcus aureus*
Jedi (J^2) 10^{-3}	*Micrococcusluteus*
Opa Eyin(O^1) 10^{-5}	*Mycobacterium lactum*
Opa Eyin((O^2) 10^{-3}	*Salmonella paratyphi*
Typhoid Asapo(Ta^1) 10^{-5}	*Citrobacter freundii*
Typhoid Asapo (Ta^2) 10^{-3}	*Clostridium sporogenes*
Malaria Asapo (Iba)(Ma^1) 10^{-3}	*Staphylococcus aureus*
Malaria Asapo (Iba) (Ma^2) 10^{-5}	*Lactobacillus casei*
Agunmu Asapo (Aga^1) 10^{-3}	*Cellulomonas biazotea*
Agunmu Asapo (Aga^2) 10^{-5}	*Micrococcus luteus*
Afato Asapo (Afa^1) 10^{-3}	*Sarcina flava*
Afato Asapo (Afa^2) 10^{-5}	*Bacillus subtilis*

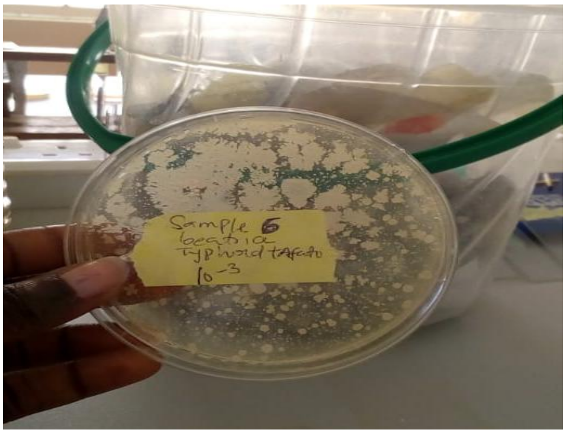

Plate 1. Plates showing the Total Bacteria count (TBC) of isolates from Malaria (iba) from Commercially Sold Herbal Cocktail samples

Plate 2. Plates showing the Total Bacteria count (TBC) of isolates from typhoid Asapo from Commercially Sold Herbal Cocktail samples

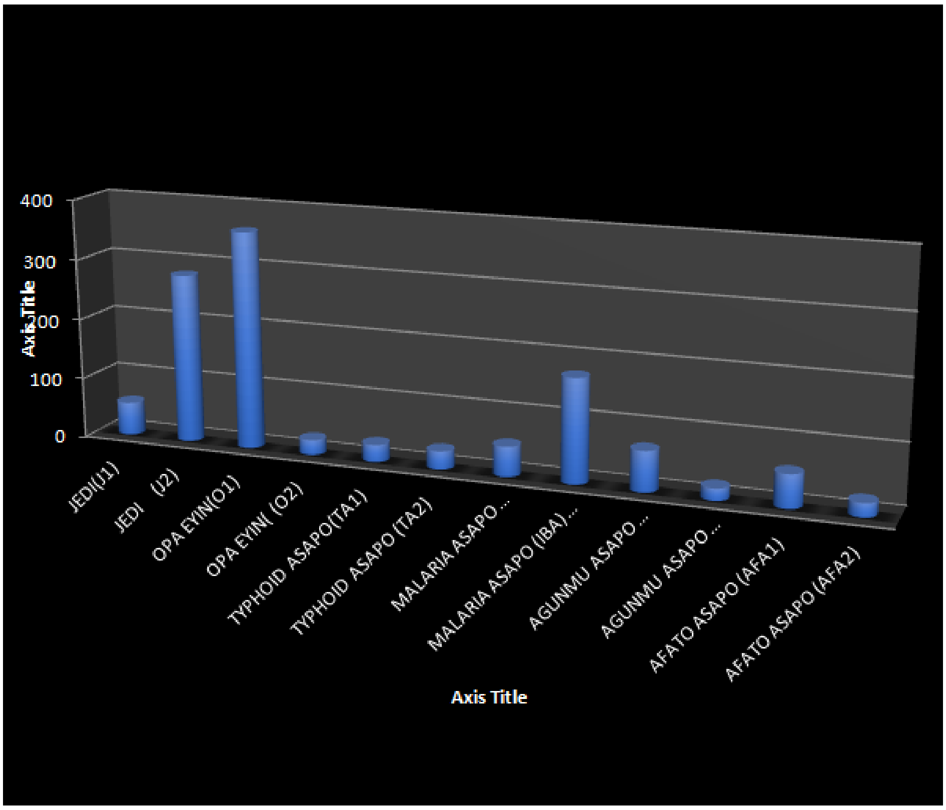

Fig. 1. Total Bacteria Count (TBC) of samples from commercially sold herbal cocktail

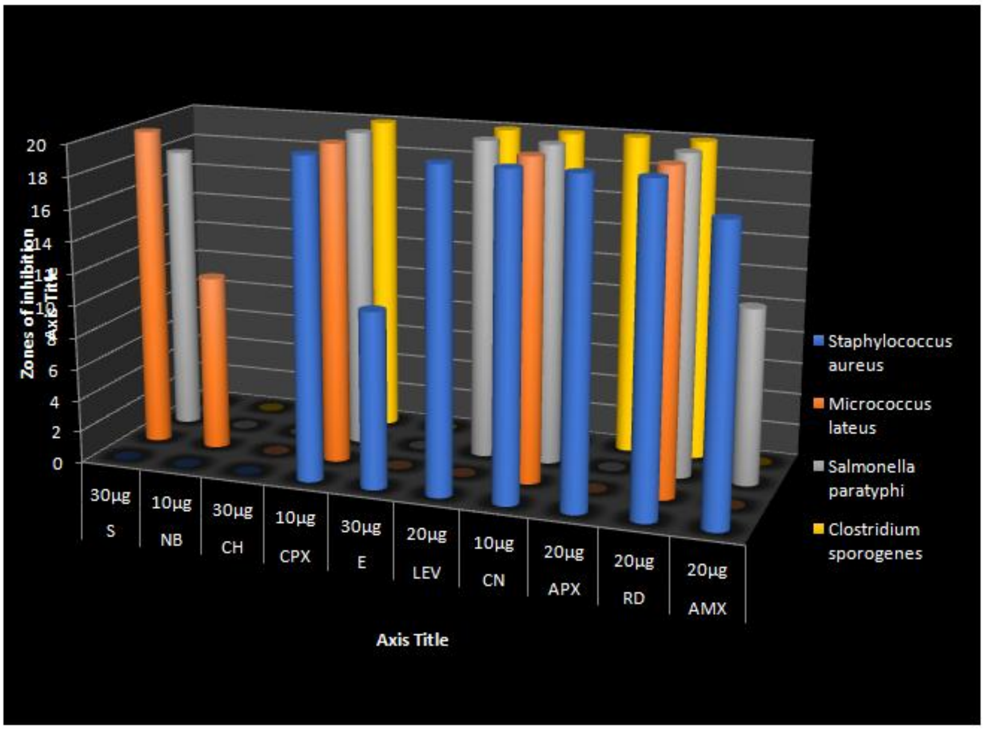

Fig. 2. Antibiotic susceptibility of selected isolates from Commercially Sold Herbal Cocktail samples against specific antibiotics

Fig. 1 showed the antibiotic susceptibility of *Staphylococcus aureus, Micrococcus luteus,* and *Clostridium sporogenes* isolates against specific antibiotics. They are 100% sensitive to Ciprofloxacin (CPX), Gentamicin (CN), and rifampicin (RD).

The antibiotic susceptibility of *Salmonella paratyphi* was depicted in Fig. 3. It can be seen that *Salmonella paratyphi* showed resistance t to the entire antibiotic used except for Streptomycin (S), Ciprofloxacin (CFX), Augmentin (AU), and Septrin (SXT).

The growth dynamic and killing time of bacteria isolates from Commercially Sold Herbal Cocktail samples against specific antibiotics. At wavelength, 480λ was shown in Figs. 4 and 5 respectively. In the growth dynamic of bacteria, the addition of ciprofloxacin at 48hrs using an ultra-violent spectrophotometer was observed. It was discovered *Salmolnella parathyhi* has the highest growth rate of 0.991λ and *Staphylococcus aureus* had the lowest death rate of 0.062λ. at 0hr. At 0hour, *C. sporogenes* has the lowest growth rate after the addition of antibiotics, *Staphylococcus aureus* has the highest growth rate i.e the antibiotics had little effect on the organism unlike the other organism after 84th hours of reading, *Micrococcus luteus* has the highest rate of 0.236λ.

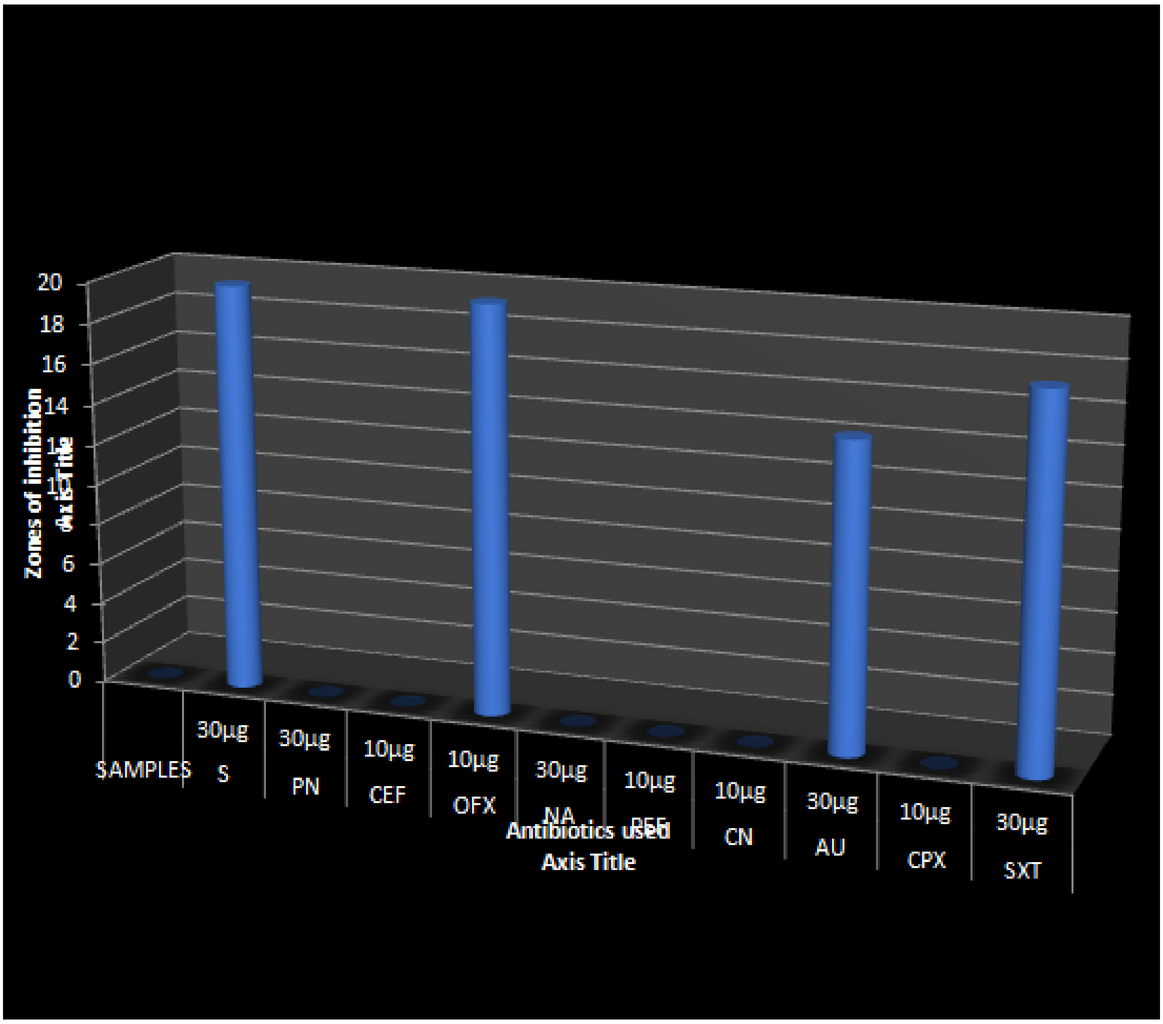

Fig. 3. Antibiotics susceptibility of Resistant *Salmonella paratyphi* from Commercially sold Herbal Cocktail samples against specific antibiotics

KEY:Tarvid (OFX), Reflacine (CEF), Augmentin (AU), Gentamicin (CN), Streptomycin (S), Cepofex (CEP), Nalidixic acid (NA), Septrin (SXT), ampicilin (PN), Ciprofloxacin (CPX), Norfloxacin (NB), Amoxicillin (AMX), Rifampicin (RD), Erythromycin (E), Chloramphenicol (CH), Ampiclox (APX) and Levofloxacin (LEV)

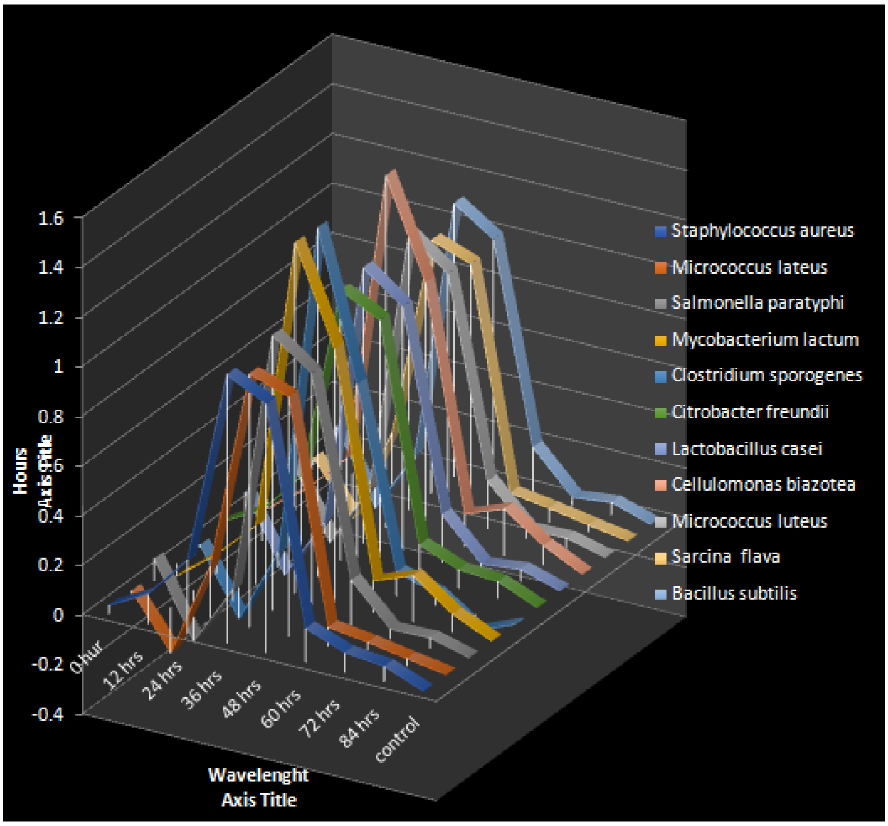

Fig. 4. UV-VIS spectrophotometer (Visible (200 ~ 760nm),To determine Growth dynamics of Isolated bacteria from Commercially Sold Herbal Cocktail

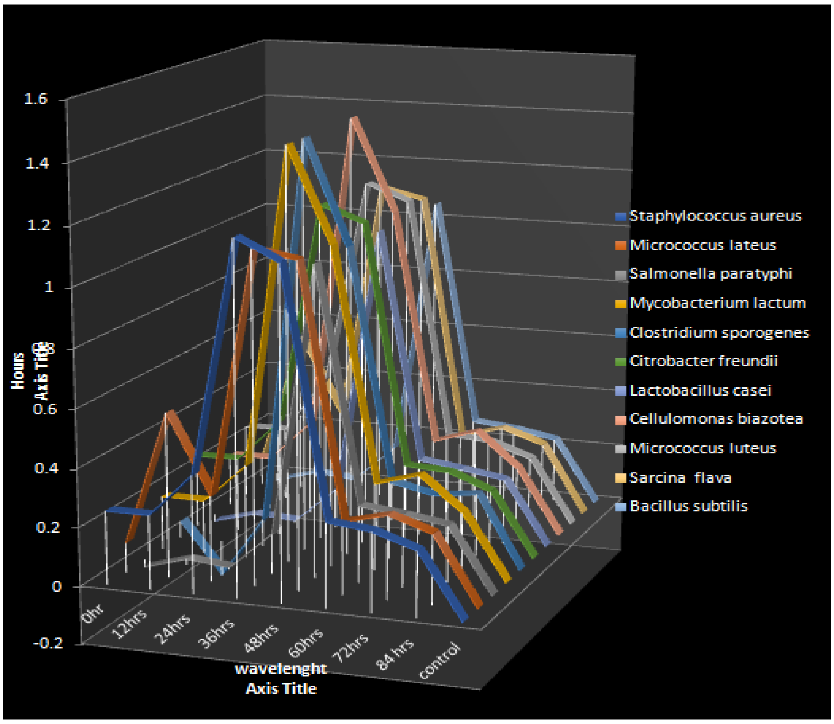

Fig. 5. UV-VIS spectrophotometer (Visible (200 ~ 760nm).), to determine the Killing kinetics of Isolates from Commercially Sold Herbal Cocktail

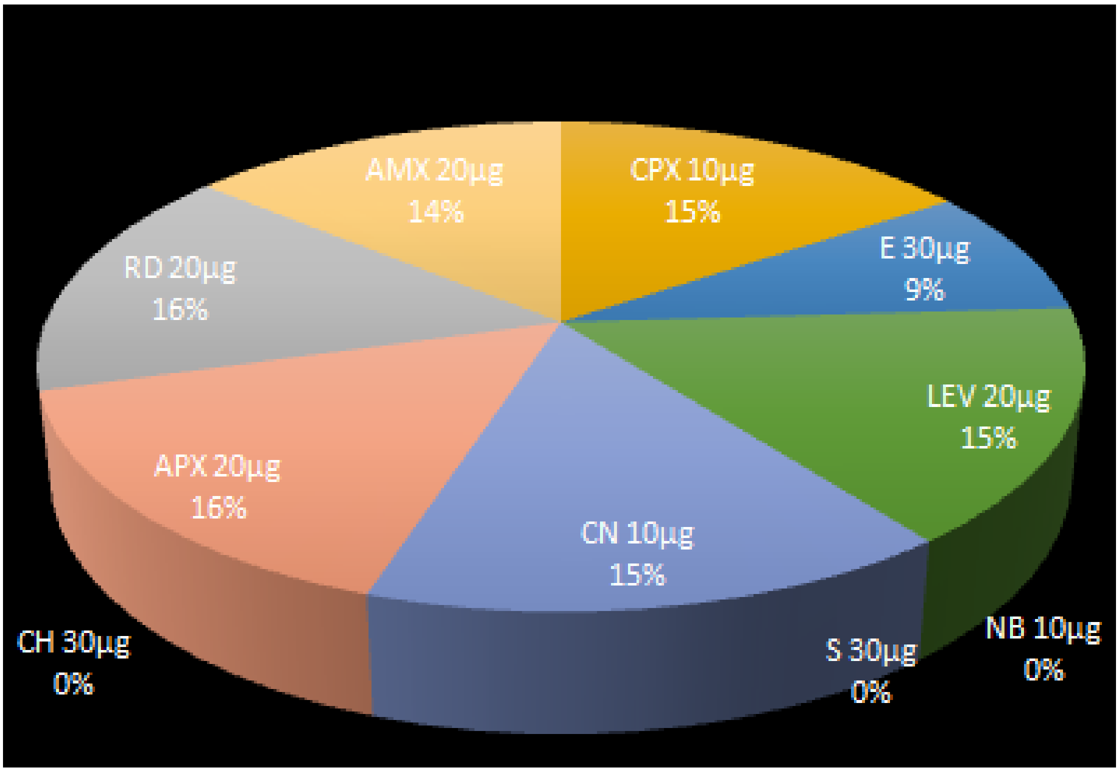

Fig. 6. Percentage frequency inhibition of selected antibotic against staphylococcus aureus isolate from commercially sold herbal cocktail

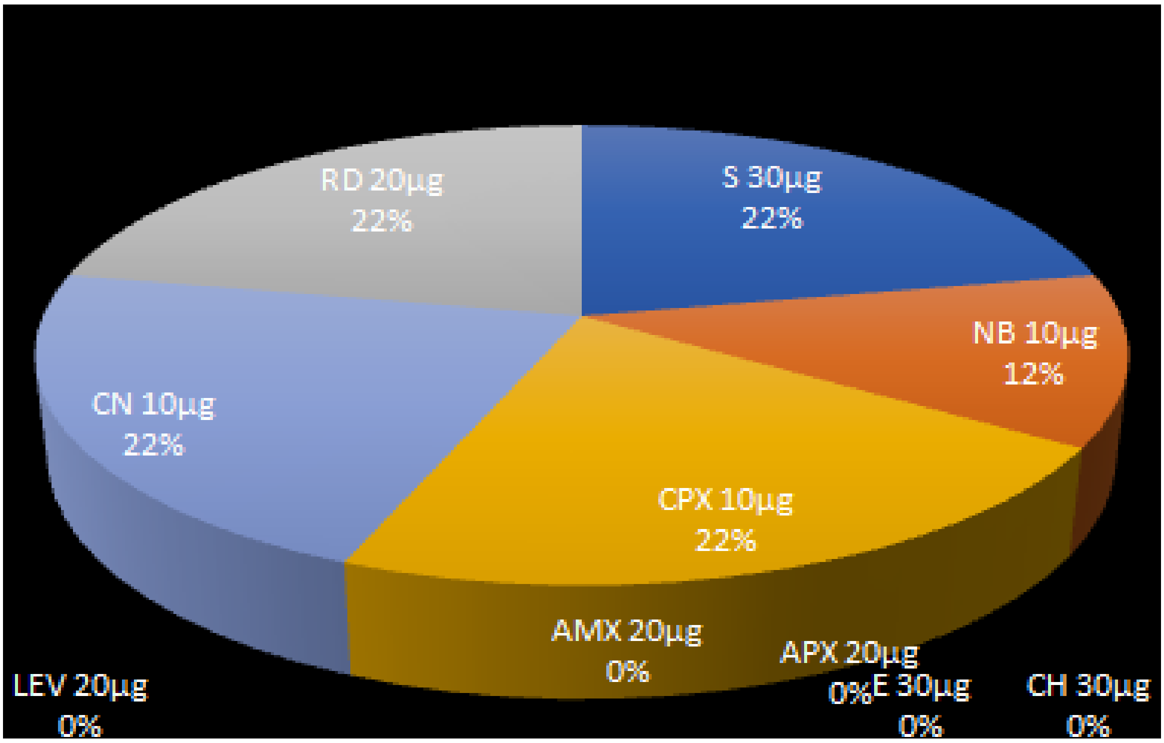

Fig. 7. Percentage frequency inhibition of selected antibotic against micrococcus lateus from commercially sold herbal cocktail

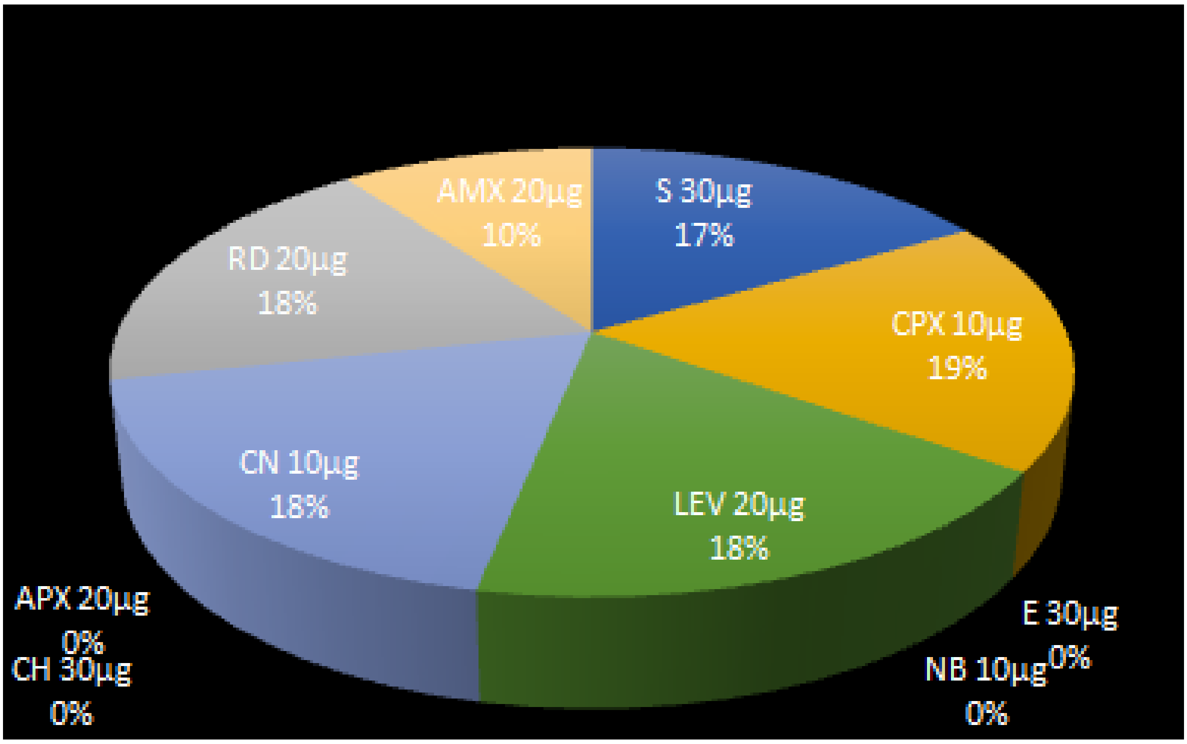

Fig. 8. Percentage frequency inhibition of selected antibotic against salmonella paratyphi from commercially sold herbal cocktail

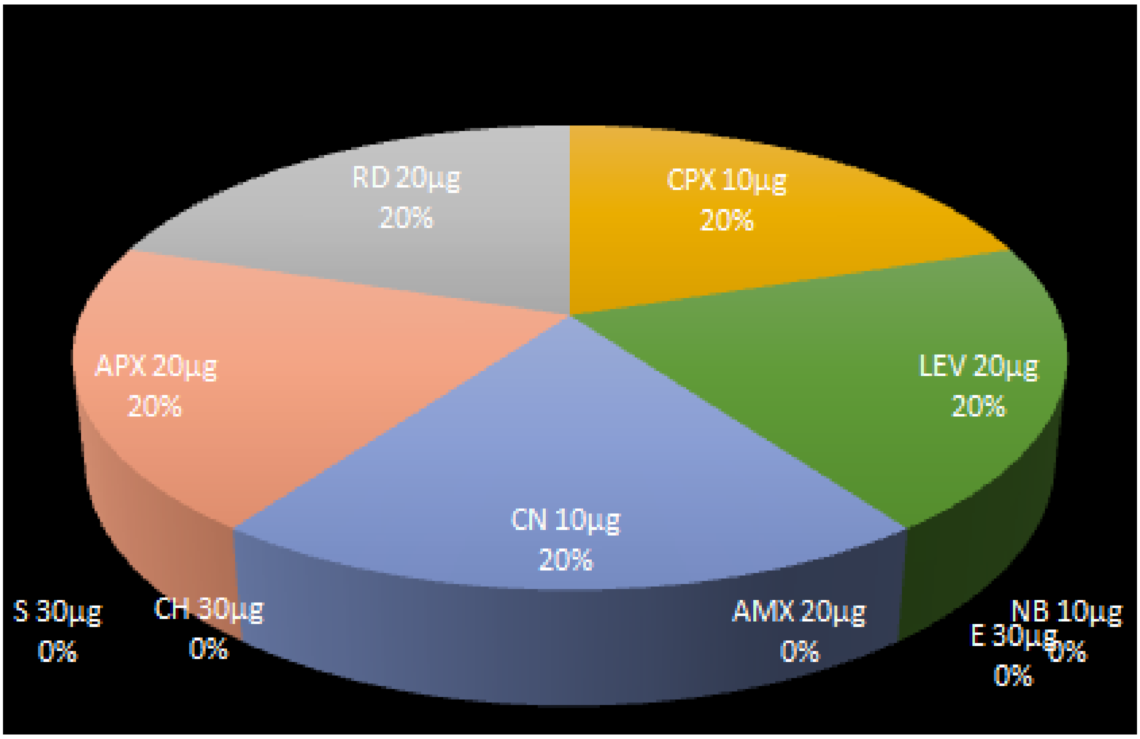

Fig. 9. Percentage frequency inhibition of selected antibotic against clostridium sporogenes from commercially sold herbal cocktail

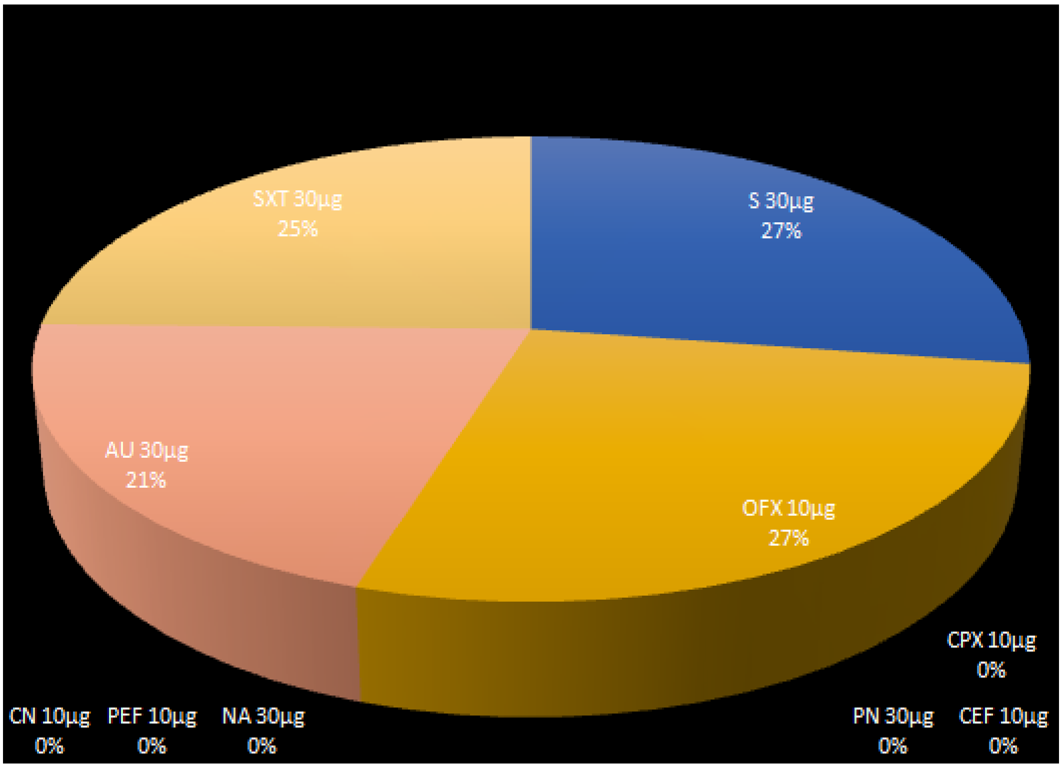

Fig. 10. Percentage Frequency Inhibition of Selected Antibiotics against *Mycobacterium lactum* From Commercially Sold Herbal Cocktail

4. DISCUSSION

The basic objectives of this research is to determine microbiological analysis, quality, and safety evaluation of commercially sold herbal cocktails from local herb sellers within Akoko southwest, Nigeria. There were indications that the commercially sold herbal cocktail were consumed by most, market people, taxi drivers, motorcycle riders and dwellers in Ikare and Akungba Area in Ondo State, Nigeria. The commercially sold herbal cocktails are contaminated even from production, with major health implications on the consumers. Herbs could easily be contaminated with multiple pathogenic bacteria during their growth, harvest, processing, and or distribution phase [22].

According to the WHO report, there is widespread availability and usage of herbal preparations by a large percentage of persons in many developing countries like Nigeria, etc [23]. Herbal medicines may harbor various hazardous microorganisms this is because the herbs are made from trees and plants which have microorganisms adhered to their stems, barks, leaves, flowers, fruits, and roots. Though these microorganisms exist in their natural environment and are normal floras of the tree. This contamination may also be injected to the consumer through the herbal practitioner during preparation, production, and packaging; this may constitute a lot of health hazards.

The results of this study showed that the total bacteria count of the herbal cocktail studied exceeded the WHO maximum limit. The highest bacteria count was seen in Opaeyin and Jedi herbal cocktails with counts of 360 and 280 respectively. This can be a result of poor hygiene practice in the preparation of the herbal cocktail or contaminant from the site of collection and even the stage of distribution. While the least count was seen in the herbal sample of Afato herbal cocktail. This result is in agreement with [24], who worked on in vitro pharmacological and synergistic effects of herbal concoctions sold in GaMaja, Limpopo province, and reported that bacterial species in concoctions were numerous to count. In Lagos, Nigeria, there are several commercially available herbal cocktails that were screened for microbial contamination as reported by [25].

The types of microorganisms isolated from the herbal cocktail which was reported in this study include *Streptococcus lactis, Staphylococcus aureus, Escherichia freundii, Micrococcus luteus, Salmonella paratyphi, Bacillus subtilis, Lactobacillus casei, Sarcinaflava, Mycobacterium lactum, Chromobacterum violaceum, Cellulomonas biazotea,* and *Clostridium sporogenes*.

The result of this study shows that some isolates such as *Salmonella paratyphi, Bacillus subtilis, Chromobacterum violaceum, Cellulomonas biazotea,* and *Clostridium sporogenes* were found to be be motile. Motility was observed as cloudy growth or protrusions moving away from the stab line in the tube. Motile bacteria are more damaging, owing to their capability to colonize cells and propagate through vast host cells, tissues, and vital organs [26]. The intake of these herbal cocktails suggests that consumers would be predisposed to bacterial infections that would effectively deteriorate their health status and maybe more debilitating to immune-compromised consumers and cause more dangers to their health. It is therefore appropriate and essential that more effective sterilization methods be used to ensure the purity of these herbal products. This is the reason Ultra-violet light from spectrophotometer were experiment during the course of this research work and the result gathered were tremendous and scholastic.

Some of the bacteria isolated in this research were *Staphylococcus aureus*, *Bacillus subtilis*, *Salmonella paratyphi* which were in agreement with studies by [27] and [28] all of which are naturally occurring in soil and water habitats. Their occurrence, well stated in the results, was probably due to their presence on plant parts and in the soil as their natural habitat [29] reported that the presence of these microorganisms in the herbal mixtures signify the substandard processing steps in the production of the herbs. Another study that examined the microbial quality of 79 medicinal herbs collected from the Egyptian market detected *Salmonella* spp. in 22.78% of the samples [30]. This result has shown that unregulated herbal medicines marketed in Nigeria are highly contaminated with microorganisms, some of which are pathogenic compared to the regulated ones. Such unregulated medicinal products may facilitate the transmission of communicable diseases in the population and therefore present a public health problem. Most of the herbal medicines analyzed in this study would be consumed at room temperature and do not get heated to above 60^{o}C before consumption, thereby increasing the risk of food-borne infections.

The growth dynamics and killing rate of all isolates revealed that all isolates drastically multiply from zero hours to the 4th measurement which could be referred to as the optimum growth stage. Growth begins to decline starting from the fifth measurement up to the eighth measurement, this decline is related to the bacteria growth curve, and this phase is known as the death phase where there is not enough nutrients for this isolated organism to survive.

The addition of Chloramphenicol deduced that there was an immediate gradual decrease in the growth of the bacteria which indicates that chloramphenicol has added to the susceptibility effect on the growth of all isolates [13,14,31]. The addition of antibiotics to the isolated organisms at the 84th-hour speed up the death rate of the isolates from commercially sold herbal cocktail between 450-480nm wavelengths. If the water used for the production of the herbal cocktail is sterilized before and after use at 450-480nm wavelength, the lowest death rate was 0.062nm. at 0hr and highest at 0.236nm at 84th hour. Water for used production of herbal cocktail needed Prolong hours of exposure to Ultraviolet rays of light is necessary, to reduce microbial load drastically, with a great effect on the quality and safety of commercially sold herbal cocktail.

5. CONCLUSION

This present study indicated varieties of microorganisms present in various herbal cocktails obtained from Ikare and Akungba Akoko of Ondo State, Nigeria, which could have resulted from contaminated soils, plants, and products, preparation processes, quality of water, containers, and processing equipment as well as distribution to consumers. However, these microorganisms exhibit multi-resistance to many antibiotics. Since herbal cocktails are mainly prepared for human consumption, there is a very high chance of passing the antibiotics resistant microorganisms into the human ecosystem. This poses a great danger to human health. Nigerian populate should be mindful of the inherent danger in drinking this so-called herbal cocktail called 'ASAPO' to avoid major health hazards and inherent diseases pandemic that may emanate from this herbal cocktail. Herbal cocktail is not bad for consuption but more hygienic practice should be employed during its preparation, production, and distribution to the general populate in South western part of Nigeria.

6. RECOMMENDATION

i. This study, therefore, suggest that proper hygienic conditions should be maintained in all preparation processes starting from plant collection, processing, packaging, and storage.
ii. Awareness should be created among the producers of the herbal concoction about the effect of consuming contaminated products.
iii. There is a need for mass education to enlighten the public on excessive consumption of herbal cocktails since many microorganisms isolated from this study are resistant to commonly used antibiotics.
iv. Also, herbal practitioners should be encouraged to send their products regularly to laboratories for quality assessment to ensure consistency and quality before marketing.
v. There is consequently a need to extend government regulation to herbal medicinal products to ensure that their processing, preparation or manufacture complies with Good Manufacturing Practices, and thus lessen risks to consumers and patients.

The need for constant monitoring and quality control of herbal medicinal products manufactured, sold, advertised, and used in Nigeria cannot be over-emphasized. As herbal medicinal products are complex mixtures that originate from biological sources, great efforts are necessary to guarantee a constant adequate quality. By carefully selecting the plant material and a standardized manufacturing process, the pattern and concentration of constituents of herbal medicinal products should be kept as constant as possible as this is a prerequisite for reproducible therapeutic results. Quality has to be built into the whole process beginning from the selection selection of propagation material to the final product reaching the consumer.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

NOTE

The study highlights the efficacy of “herbal medicine " which is an ancient tradition, used in some parts of Nigeria. West Africa. This ancient concept should be carefully evaluated in the light of modern medical science and can be utilized partially if found suitable.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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