Original Research Article

Virulence response of *Escherichia coli* to different concentrations of combined herbal drugs

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ABSTRACT

Background: The use of combinational approach in chemotherapeutic management has proven more effective against infectious disease and lower resistance development but the untoward effect of this is yet to be explored for alternative medicine.
Aims: This study aimed to study the effect of a combinational approach of herbal drugs on *Escherichia coli* response.
Methods: *E. coli* was treated in different concentrations of combined herbal drugs (Beta herbal drugs and Deep root herbal mixture – BD) and (Beta herbal drug and Goko cleanser – BG). The different concentrations of the mixtures employed were: 33.33 %, 11.11 %, 3.7 %, 1.23 % and 0.41 %. The bacteria concentration of 10^3 CFU/ml was treated in the different concentrations of the herbal drugs. The growth response of the cultures were analyzed at 24 and 48 hrs. The antibiotic sensitivity of the bacteria exposed to the herbal drugs were measured against perfloxacin (PEF), ciprofloxacin (CIP), streptomycin (S), and septrin (SEP).
Results: The growth response curve showed growth of *E. coli* peaked in the lower concentrations but levelled down in the higher concentrations. 24 hr growth conditions showed much higher growth level than 48 hrs. Higher concentrations of BG drug combination showed higher zones of clearance and only one concentration showed resistance: 0.41 % (CIP). There was no unique pattern seen in the sensitivity of *E. coli* treated with BD however, there was four (4) cases of complete resistance: 0.41 % (PEF), 1.23 % (PEF) and 11.11 % (CIP and SEP).
Conclusion: The combined herbal drugs do not completely eliminate *E. coli*. However, some concentrations of the combinations demonstrated complete resistance to the modern antibiotics which shows that these locally made antimicrobial usually confer resistance to *E. coli* as illustrated using the sensitivity test.

Keywords: Herbal drugs, drug resistance, combinational therapy, antimicrobial sensitivity

1. INTRODUCTION

The evolution of microorganism has also brought about their resistance to antimicrobial therapy, this resistance has plagued the world of clinical medicine worldwide and its emergence is a problem that has to be tackled with immediate effect globally. An organism is said to be resistant when it is able to resist the effect of antimicrobial agent i.e. the causative

pathogen of the infection/disease is not killed or its growth is not inhibited. Infections with antimicrobial resistance are usually difficult to treat and require more cost for treatment [1].

Antimicrobial is a compound name which encompasses resistance caused by different microbial agents which includes: bacteria, fungi, viruses and some protozoans, these antimicrobials are used to kill or inhibit the growth of infectious microorganisms. Microorganisms that develop antimicrobial resistance are sometimes referred to as "superbug". Superbug renders antimicrobial therapy ineffective which leads to persistence of the infection and has a high risk of spread of resistant gene to new organism [2]. The rapid and sudden uprising of resistance of microorganism is a worldwide threat and it has disrupted the effects of antimicrobial which has been used for treatment against infectious disease and also saved lives [3]. One major factor which has enhanced the spread or propagated antimicrobial resistance has been attributed to over-use of antimicrobial therapy as well as failure of pharmaceutical companies to produce new antimicrobial therapy [4]. Antimicrobial resistance (AMR) has posed to be more in developing countries and under developed countries this is due to the fact that they are more prone to infectious diseases which have led to higher consumption of medicine and at such disproportionately higher incidence of inappropriate use of antibiotics and greater levels of resistance compared to developed countries. This has been noted to be of great threat to humans globally [5]. Globally, antimicrobial therapy has been of tremendous help to the world of medicine this is a fact that cannot be overemphasized [6]. This has been accomplished by reducing mortality and morbidity rate in infected patients [7]. However, the fight has not been won completely as statistics carries that infectious diseases still cause about 20% of death globally equating to about 11miilion deaths per annum. This is also due to the emergence of antimicrobial resistance. Increased antimicrobial resistance is the major cause of severe infections, complications, longer hospital stays and increased mortality and at such understanding the mechanism of resistance and the type of organism is the first step in tackling this serious medical dilemma.

Amidst all antimicrobial resistance (AMR), antibiotics (antibacterial) resistance is more common because bacteria are ubiquitous and antibiotics are the most common medications used clinically by health professionals to tackle the spread of bacteria. Bacteria is said to be resistant to an antibiotic when the antibiotic can no longer kill or inhibit the growth of the bacteria effectively and at such the pathogen continues to multiply in the presence of high therapeutic dosage of the antibiotic [2]. Resistance to antibiotics has predominantly been the most reported case of antimicrobial resistance and has been a problem in hospital settings, recent research carried out by national collaboration center for infectious diseases in 2010 has shown that resistant microorganism have also been observed in patients in primary care. According to the centers for disease control and prevention in 2012, some diseases are mostly associated with antimicrobial resistance (AMR) in primary health care and they include; tuberculosis, gonorrhea, typhoid fever and group B streptococcus [1]. As in the case of other AMRs, research shows that antibiotics resistance is due to inappropriate use i.e. prescribing antimicrobials when not necessary or prescribing a broad spectrum agent when a narrower spectrum agent would have also been useful [8].

Herbal medicines are drugs gotten from plants or its extract, which contain therapeutic substances. They are usually used in empirical treatment of ailments. China has history of 500years in use of herbal medicines. It is described in the holy bible about medicinal plant species, example; myrrh and frankincense, which were reported to have antiseptic and healing properties. Herbal medicine is becoming more popular not only in developing countries but also in developed countries. Many studies have been conducted across the globe to prove or find the antimicrobial properties of herbal drugs [9].

The rate of occurrence of antimicrobial resistance has increased exponentially. This is due to poorly recognized mechanism of antimicrobial resistance. Some researchers proposed that locally made drugs do not confer resistance to microorganism, while some opposed that proposition. It is believed that herbal drugs harbor drug resistant microbiota which can lead to ingestion of these resistant strains. The aim of the study was to investigate whether the combination of two locally made drugs confer some level of resistance to opportunistic bacteria (*E. coli*). To determine the growth response of the test organism to two combined locally made antimicrobial.

2. MATERIALS AND METHODS

2.1 Herbal Drugs

The herbal drugs used were Beta herbal mixture (B), Deep root herbal mixture (D), Goko cleanser (G). These drugs were purchased from Mile 3 Market, Porthacourt, Nigeria. 5 ml of beta and deep root (BD) each was dispensed into a sterile container. Again, 5ml of beta and goko cleanser (BG) each was dispensed into another sterile container. Each of the drug containing vessels was shaken gently in order to mix the content.

2.2 Test Organisms

Escherichia coli strain used in this research was purchased from Lahor Research Laboratory, Benin City, Edo State. The identity of the microorganism was subjected to confirmatory testing such as MacConkey agar, indole and Grams stains according to Chesbrough [10]

2.3 Media Preparations

Tryptone soy agar (TSA) and tryptone soy broth (TSB) were prepared according to the manufacturer's instructions and autoclaved at 121 °C for 15 minutes. The prepared media were allowed to cool to about 47 °C, and 20 ml was poured aseptically into sterile Petri dishes for TSA. The plates were allowed to solidify at room temperature and stored in 4 °C for subsequent use, while the TSB was stored at 4 °C in a refrigerated environment.

2.4 Growth Response to the Herbal Drugs

The growth response was carried out by using the spectrophotometric method. In the spectrophotometric method, overnight inoculum was serially diluted to 10^8 and treated with different concentrations of BG and BD serially diluted in different concentration of the overnight inoculums, and incubated for 18 hrs at 37 °C. The optical densities of the various concentrations were obtained using spectrophotometer at a wavelength of 600 nm.

2.5 Antimicrobial Susceptibility Testing of the Herbal Drug-Treated *E. coli*

Overnight inoculums were made and the optical density was read using spectrophotometer at 600 nm. The overnight inoculum was serially diluted to 10^8. From this starting concentration 2 ml of bacteria, aliquot were placed in five different bijou bottles. Five concentrations of the herbal drug were made by serial dilution using TSB medium as the diluents. The concentration of the drug from the first to fifth Bijou bottle were 33.33 %, 11.11 %, 3.7 %, 1.23 % and 0.41 % respectively. All the bottles were incubated at 37 °C and their optical densities were read after 24 hrs incubations to check the response to the BG and BD. After 24 hrs, from the different bijou bottles, 10 µl of incubated TSB containing the bacteria were spread over the entire surface of TSA using a sterile spreader, and the antibiotic discs

(ciprofloxacin, pefloxacin, streptomycin and septrin) were placed on the five concentrations of BG and BD respectively. A control experiment of *E. coli* not previously exposed to BG or BD was performed in the same condition as those previously exposed to BG and BD. The sensitivity plates were incubated at 37 °C for 24 hrs and the zones of inhibition were measured in mm.

2.6 Data Analyses

The data obtained in this study were performed in duplicate and presented as graph using GraphPad Prism version 8.0.

3. RESULTS

3.1 Growth Responsiveness to Local Drugs

Figure 1 depicts the growth response of *E. coli* to different concentrations of herbal drugs. Some level of clearance in a broth culture of the test organism (*E. coli*) was seen. This level of clearance is directly proportional to the concentration of the drug, the higher the concentration, the higher the level of clearance. Unfortunately, these locally made drugs even in their combined state and highest concentrations; could not produce complete inhibition of *E. coli*. One can say that herbal antimicrobials do not kill the organism in its entirety. From Figure 1A, both 24 hrs and 48 hrs, was shown that there are some levels of growth at about 3 % concentration, though the growth level for the various concentrations varies**.** The diagram showed that the higher the concentration of the drug, the lower the absorbance. Also, it was seen that the 24 hrs OD reading had a higher value than that of the 48 hrs.

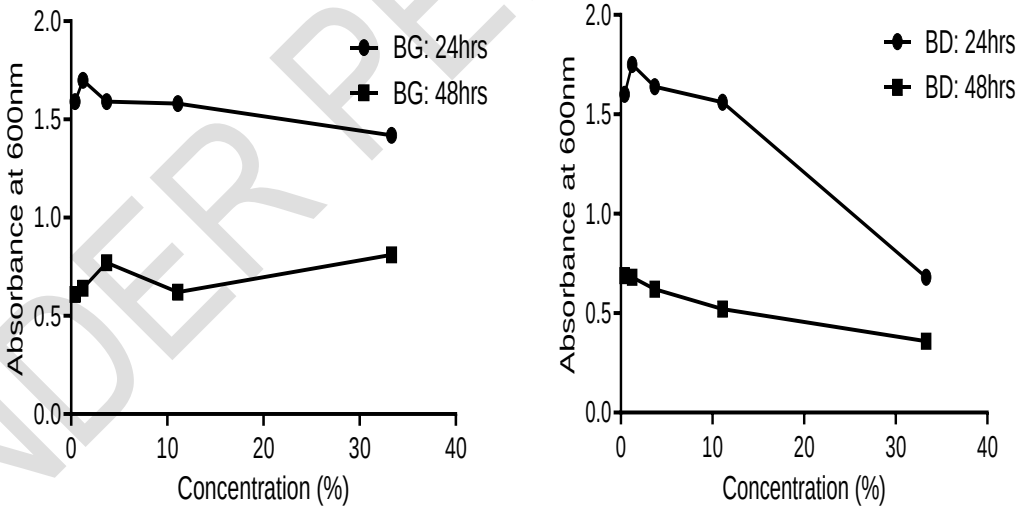

Fig. 1. Growth Response of *E. coli* to different Concentrations of Herbal Drugs.
A: Beta drug and Goko cleanser drug , B: Beta drug and deep root drug

3.2 Antimicrobial Sensitivity of BG-treated *E. coli*

Table 1 zones of clearance by BG-treated *E. coli.* Pefloxacin produced a higher zone of clearance in the control (28 mm), which was higher than the other local drug-treated organisms. Each of the various concentrations has varying diameter of the zone of clearance, which are lower than the control. Ciprofloxacin showed a nearly similar but different result compared to that of Pefloxacin. Here the first concentration and second concentration had the same diameter of zone of clearance with the control. It is also seen that the forth concentration exhibited resistance to the modern drug. In streptomycin, the third concentration had the same zone of clearance with the control, while others had varying zones of clearance. There was no complete resistance of the test organism to streptomycin. Septrin showed no complete resistance to the test organism, although there were varying levels of resistance to the antibiotic.

3.3 Antimicrobial Sensitivity of BD-Treated *E. coli*

Table 2 shows the zones of clearance by BD-treated *E. coli*. The Pefloxacin drug had very different pattern of clearance with BD-treated *E. coli;* the third concentration and the fifth concentration showed no susceptibility to the modern drug, while other concentrations had varying levels of clearance. Ciprofloxacin showed quite a remarkable pattern of clearance, with the second concentration showing resistance to the modern drug. The third and fifth concentration had a higher zone of clearance compared to the control, while the first concentration (0.333) had a lower zone of clearance than the control, as shown in table 2. Just like Ciprofloxacin, the third and fifth concentrations for Streptomycin had a higher zone of clearance compared to the control. Other concentrations had varying zones of clearance but lower than the control's zone of clearance. Septrin had a different pattern of clearance, the second concentration produced resistance to the modern drug. Other concentrations had differing concentrations which are different from and lower than that of the control.

Table 1. Zones of Clearance by BG-treated *E. coli*

Herbal drug Concentration (%)	Zone of Inhibition (mm) PEF	CIP	S	SEP
0.333	24	24	18	18
0.111	19	19	16	14
0.037	17	24	24	16
0.012	18	14	18	14
0.004	17	0	11	12
-Ve Control	28	28	27	28

**PEF – Perfloxacin, CIP – Ciprofloxacin, S – Streptomycin, SEP - Septrin*

Table 2. Zones of Clearance by BD-treated *E. coli*

Herbal drug Concentration (%)	Zone of Inhibition (mm) PEF	CIP	S	SEP
0.333	18	10	1	18
0.111	24	0	14	0
0.037	25	25	25	16
0.012	0	19	15	16
0.004	0	23	23	18
-Ve Control	28	28	27	28

**PEF – Perfloxacin, CIP – Ciprofloxacin, S – Streptomycin, SEP - Septrin*

4. DISCUSSION

Herbal antimicrobials of a truth have some level of potency in the test organism. From the result, it was seen that these locally made drugs can confer some level of resistance, even with a combination of the herbal drug. Khan et al. [11] reported that many clinical and non-clinical bacterial isolates were sensitive to the herbal drug called *A. nilotica.* Although another study has shown that some of the isolates like *P. aeuroginosa, E. coli,* etc. which were isolated from community acquired infection, were resistant to herbal drugs [9].

The effect of the interactions of herbal drugs can lead to three results: synergy, antagonism or no reaction on the microorganism [12]. The growth curve of *E. coli* in the herbal mixture showed that there was a gradual increase in the number of bacteria which later dipped into a decrease thereby depicting a sigmoid curve growth. This is similar to the illustration given by Bhardwaj et al. [12].

From the sensitivity result, it was seen that there is a high zone of inhibition in the negative control (organism not treated with locally made antimicrobial) as against those exposed to the herbal drug, prior to treatment with modern drugs. The details of the sensitivity result were as follows; Pefloxacin: the control showed a high zone of clearance, while the diameter of the zone of clearance is reduced in the test organism which was initially exposed to the locally made antimicrobial. This result is replicated in treatment with; Ciprofloxacin, Streptomycin, and Septrin, as can be seen in the graphs given above. According to Singh *et al*., 2016, both reference strains used in their study were sensitive to all 10 herbal antibiotics used in the study, however, none of the strain had resistance to mercuric chloride but zone of inhibition varied from 6 mm – 35 mm for different bacteria, all strain showing narrow zone of inhibition to mercuric chloride disc belong to the *Enterobacteriacae* family. This is evidential of the fact that in as much as herbal drugs confer some level of activities, it does not entirely kill the bacteria, also, it agrees to the fact that bacteria which are exposed to locally made drugs develops resistance for the contemporary antimicrobial drugs. Again, it is also known from research that some microbes that inhabit on fruits or leaves with antimicrobial properties, are resistant to modern antimicrobial, this might be due to their adaptive nature towards antimicrobial activities.

Therefore, patients are advised to maintain treatment with the modern drug, because it has more controllable outcomes than the locally made drugs, thus, can reduce the rate of antimicrobial resistance of microorganisms. It is imperative to conduct research on how to modify these locally made drugs, owing to the fact that they have some level of potency on the test organism. Likewise, research should also be conducted on the mechanism by which these locally made drugs confer resistance to microbes against modern drugs. This could ultimately reduce the level of multidrug resistant patients. Again, the local drug producers should work in synergy with the conventional scientists to verify the efficacies and potencies of the drugs.

4. CONCLUSION

The data obtained from the research showed that locally made antimicrobials, though have some level of potency and effectiveness, they do not confer complete elimination of the test organism. Thus, an individual infected with the *E. coli*, on treatment with the local antimicrobial, will not be completely treated. More importantly, when the test organism was exposed to the local drugs, the organism develops some levels of resistance to the modern drugs. Some even demonstrated complete resistance to the modern drugs. This is evidential that these locally made antimicrobial usually confer resistance to *E. coli* as illustrated using the sensitivity test.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. 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.

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