



Antibiotics Sensitivity of *Escherichia coli* to Different Concentrations of Combined Herbal Drugs

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Authors' contributions

This work was carried out in collaboration among all authors. Author MTP designed the study, performed the statistical analysis. Author SOC performed the experimental activities, wrote the protocol and wrote the first draft of the manuscript. Authors MTP, NCU and ASE managed the analyses of the study. Author SOC managed the literature searches. All authors read and approved the final manuscript.

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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: 33.3%, 11.1%, 3.7%, 1.2% and 0.4%. The bacteria concentration of 10³ 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 hr. Higher concentrations of BG drug combination showed higher zones of clearance and only one concentration showed resistance: 0.4% (CIP). There was no unique pattern seen in the sensitivity of *E. coli* treated with BD however, there was five (5) cases of complete resistance: 0.4% (PEF), 1.2% (PEF) and 11.1% (CIP and SEP).

Conclusion: In vitro, the combined herbal drugs do not completely eliminate *E. coli*. However, some concentrations of the combinations demonstrated complete resistance to the antibiotics which shows that these locally made antimicrobial could confer antibiotic resistance to *E. coli*.

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

1. INTRODUCTION

The evolution of microorganisms 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 can 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 that 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 “superbugs”. Superbug renders antimicrobial therapy ineffective which leads to persistence of the infection and has a high risk of spread of resistant gene to the new organism [2]. The rapid and sudden uprising of resistance of microorganisms 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 the over-use of antimicrobial therapy as well as the failure of pharmaceutical companies to produce new antimicrobial therapy [4]. Antimicrobial resistance (AMR) has posed to be more in developing countries and underdeveloped countries this is since 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].

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, gonorrhoea, typhoid fever and group B *Streptococcus* [1]. As in the case of other AMRs, research shows that antibiotic 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 the extract, which contain therapeutic substances. They are usually used in the empirical treatment of ailments. 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 current study investigated goko bitters, deep root herbal mixture and beta cleanser. Some of the constituent of these extract are; *Calamus rhizome*, *Azadirachta indica* and Caramel [10]. The study aimed to investigate whether the combination of two locally made drugs confers some level of resistance to opportunistic bacteria, *Escherichia coli*.

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, Port Harcourt, Nigeria. 5 ml of beta and deep root (BD) each was dispensed into a sterile container. Again, 5 ml 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 ATCC 252922 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 [11].

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 (1×10^3 cfu/ml) and treated with different concentrations of BG and

BD serially diluted in different concentration of the overnight inoculums, and incubated for 18 hr 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 33.3%, 11.1%, 3.7%, 1.2% and 0.4% respectively. All the bottles were incubated at 37°C and their optical densities were read after 24 hr incubations to check the response to the BG and BD. After 24 hr, 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 Oxoid antibiotic discs (ciprofloxacin 10 µg, pefloxacin, streptomycin 30 µg and septrin 10 µg) 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 hr 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

Fig. 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. 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 Fig. 1A, both 24 hr and 48 hr, 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 hr OD reading had a higher value than that of the 48 hr.

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

Table 1 depicts the 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 similar but different result compared to that of Pefloxacin. In this case, the first concentration and second concentration had the same diameter of zone of clearance with the control. It is also seen that the fourth 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 on BD-treated *E. coli*. The *E. coli* treated in fourth and the fifth concentrations (1.2 and 0.4 % respectively) showed no susceptibility to the antibiotics, while other concentrations had higher levels of clearance. Ciprofloxacin showed quite a remarkable pattern of clearance, with *E. coli* treated in the second concentration (11.1%) showing complete resistance to the modern drug. third, fourth and fifth concentrations (3.7, 1.2, 0.4% respectively) had a higher zone of clearance compared to the control, while the first concentration (33.3%) had a lower zone of clearance than the control, as shown in Table 2. Similar to ciprofloxacin, the *E. coli* treated in the third concentration (3.7%) 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. For septrin, the *E. coli* treated in the second concentration (11.1%) produced resistance to the antibiotics. Other concentrations had differing concentrations which are different from and lower than that of the control.

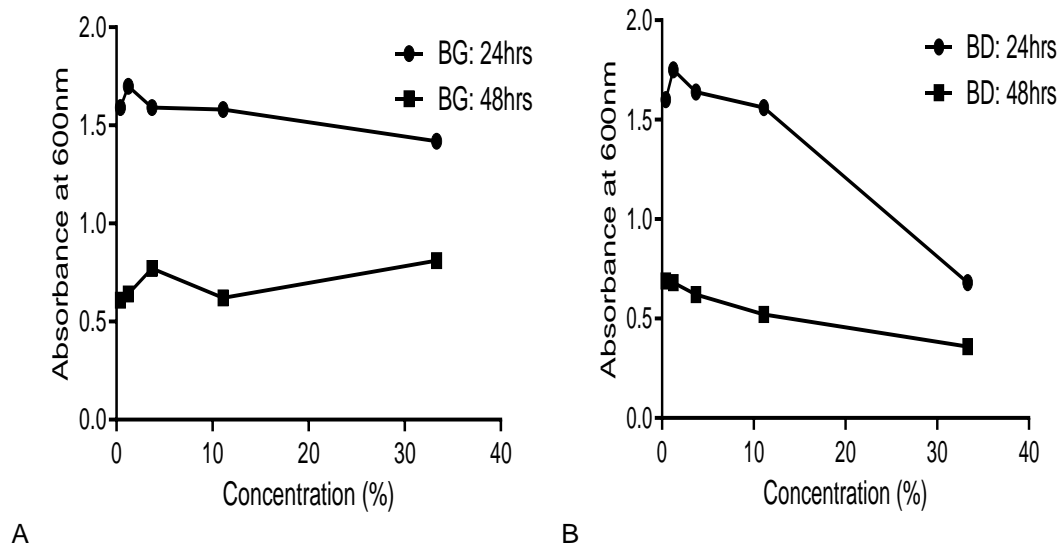


Fig. 1. Growth Response of *E. coli* to different Concentrations of Herbal Drugs
 A: Beta drug and Goko cleanser drug, B: Beta drug and deep root drug

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

S/N	Herbal drug Concentration (%)	Zone of Inhibition (mm)			
		PEF	CIP	S	SEP
1	33.3	24	24	18	18
2	11.1	19	19	16	14
3	3.7	17	24	24	16
4	1.2	18	14	18	14
5	0.4	17	0	11	12
6	-Ve Control	28	28	27	28

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

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

S/N	Herbal drug Concentration (%)	Zone of Inhibition (mm)			
		PEF	CIP	S	SEP
1	33.3	18	10	19	18
2	11.1	24	0	14	0
3	3.7	25	25	25	16
4	1.2	0	19	15	16
5	0.4	0	23	23	18
6	-Ve Control	28	28	27	28

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

4. DISCUSSION

Herbal antimicrobials possess antimicrobial potency on the test organism. From the result, it was seen that these locally made drugs could confer some level of resistance, with a combination of the herbal drug. Khan et al. [12] reported that many clinical and non-clinical bacterial isolates were sensitive to the herbal drug called *A. nilotica*. A previous study has shown that some of the isolates such *P. aeruginosa*, *E. coli*, 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 [13]. 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. [13]. The presence of *E. coli* was observed throughout the 48 hr duration of the study which implies that the combined herbal drug could not completely eliminated the bacteria. Hence, this study further studied the sensitivity response of the bacteria in different concentrations of the combined herbal drug.

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. A study showed that two reference bacterial strains 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 *Enterobacteriaceae* family [14]. This shows that although herbal drugs confer antimicrobial potential, it does not entirely kill the bacteria.

The zones of inhibition to antibiotics observed in this study was not directly proportional to the different concentrations of the herbal drugs the *E. coli* were treated in. Five (5) cases of complete resistance (no zone of inhibition) were noted. For the BG treatments, only the *E. coli* treated in 0.4% BD exhibited no zone of clearance to Ciprofloxacin. Four (4) cases of resistance were observed in BD treatments: 11.1% BD (ciprofloxacin and septrin), 1.2% BD (perfloracin) and 0.4% BD (perfloracin).

Previous studies have shown that a key bacteria adaption to adverse conditions is a modification of their response to antibiotics [15,16,17].

In the publication by Amala et al. [15], *E. coli* and *Staphylococcus aureus* isolates exposed to hydrocarbon-generated soot showed complete resistance and lowered sensitivity to antibiotics which is similar to the results observed in the current study. Monsi et al. [17] also assessed the phenotypic response of *Pseudomonas aeruginosa* to different environmental conditions such as temperature, pH and oxygen and an elevated growth and reduced antibiotic resistivity was noted. These observations are in line with those observed in this study.

5. CONCLUSION

The data obtained from this study noted five (5) resistance cases of *E. coli* after treatment with combined herbal drugs. This implies that BD and BG herbal drug combinations could cause resistance to herbal drug sensitized *E. coli*.

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.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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