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COMBINATION ACTIVITY OF ANTIBIOTICS AND HERBAL FORMULATIONS AGAINST SELECT BACTERIAL PATHOGENIC ORGANISMS

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ABSTRACT: The antibacterial activity of some commercial herbal medicine preparation in combination with antibiotics against five Gram positive and negative bacteria were studied using the agar diffusion method. The herbal medicines namely Alomo bitters, Goko cleanser, Chimason mighty and Yoyo bitters and the antibiotics; Ampicillin, Cloxacillin, Chloramphenicol and Tetracycline were tested alone against the test organisms *Pseudomonas aeruginosa, Staphylococcus aureus, Escherichia coli, Salmonella sp* and *Shigella sp.* Alomo bitters and Goko cleanser were selected and tested in combination with all four antibiotics against the organisms. The result shows that individually, both the herbal medicine and antibiotics demonstrated varying levels of antibacterial activity with recorded diameter zones of inhibition ranging from 0 to 35mm. While the combination of the two most active herbal medicines (Alomo bitters and Goko cleanser) with the antibiotics showed both a positive and negative drug-herb interactions and an indifference. Alomo bitters and antibiotics gave a 45% positive interaction, 15% negative interaction and 40% non-interaction or indifference. While Goko cleanser and antibiotics gave a 65% positive interaction, 10% negative interaction and 25 % non-interaction or indifference. This shows that herbal medicine preparations and antibiotics may be used in combination and complementary medicines in the fight against infectious microbial diseases.

Keywords: Herbal medicine, Antibiotics, Combination, Interaction

1. INTRODUCTION

The issue of antibiotic resistance among microorganisms has been in the front burner for some time now. Microorganisms have continued to evolve and evade the lethal actions of antibiotics by employing several different strategies [1]. This phenomenon has continued to rise and assume a global dimension and worldwide threat to the management of public health [2]. While bacteria and other organism's ability to survive in harsh and adverse environmental conditions are sometimes determined by their inherent genetic composition and other mechanisms, the abuse and misuse of these antibiotics by man both in human health and veterinary purposes have contributed immensely to the development and spread of antibiotics resistance [3]. However, since the infectious disease war cannot be lost to antibiotic resistant bacteria, there is the need to constantly search for other alternative strategies to stay ahead of the organisms and combat the scourge of microbial infections. Current strategies include developing new drugs, looking for new drug targets, use of medicinal plants and the use two or more antimicrobial agents in a combination therapy [4,5]. The combination of two or more antimicrobial is gaining more attention because of its potential to overcome antibiotic resistance. The combination of antimicrobials can result in a positive or negative interaction broadly classified as synergistic, additive, indifference or additive interactions [6]. These antimicrobial combinations particularly between plant derived bioactive agents with antibiotics, enhances the activity of the antimicrobials, lowers the effective concentrations of the agents and reduces the side effects of these antibiotics [7].

Several in-vitro researches have reported the use of plant extracts and antibiotics in combination with varying results. The antibacterial activity of leaves and bark extracts of *Azadirachta indica* in combination with aminoglycosides and carbapenems against *Staphylococcus aureus, Escherichia coli* and

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Pseudomonas aeruginosa was reported [8]. Ethanol bark extract and amikacin, imipenem, gentamicin against *P. aeruginosa*, *E. coli* and *S. aureus* strains was shown to have synergistic activity [9].

In addition, other in vitro studies have reported the capacity of pure compounds to enhance the activity of antibiotics. Combinations of tetracycline with baicalein (5,6,7 trihydroxyflavone) exhibit synergistic effects against MRSA [10]. Curcumin, a flavonoid isolated from Curcuma longa L., markedly reduced the MICs of the antibiotics oxacillin, ampicillin, ciprofloxacin and norfloxacin used against MRSA [11]. Allicin, antibacterial compound from garlic (*Allium sativum*), enhanced the action of cefazolin and oxacillin, against *Staphylococcus* sp. and cefoperazone against *P. aeruginosa* [12].

Despite these reports, there are still worries and concerns expressed by practitioners and patients alike in combining both antibiotics or other chemotherapeutic agents and herbal medicine preparations. Some have advised to discontinue the use of herbal remedies while administering antibiotics and vice versa. This work is therefore aimed at evaluating the outcome of an antibiotic and herbal medicine combination in vitro and as a potential strategy to overcome the issue of antibiotics resistance in bacteria.

2. MATERIALS AND METHODS

2.1 Herbal Medicine Preparations

The herbal remedies Alomo bitters, Yoyo bitters, Chimason mighty and Goko cleanser were obtained from traditional herbal medicine manufacturers in Owerri, Imo state, Nigeria. The preparations were made using extracts from *Aloe-vera, Acinos arventis, Citrus aurantifolis, Chenopodium murale, Cinnamomum aromaticum, Vernonia amydalina, Saccharum officinarum, Allium sativum, Cayanus cajan, Zingiber officionale* and caramel. These were composition of the herbal remedies as specified by the manufacturer.

2.2 Antibiotics

The following antibiotics Ampicillin, Cloxacillin, Chloramphenicol andTetracycline were used for the study.

2.3 Microorganisms

The following microorganisms were used; *Pseudomonas aeruginosa, E. coli, Staphyloccocus aureus, Salmonella* sp, and *Shigella* sp. All the bacterial strains were obtained from the medical laboratory unit of the Federal medical Centre Owerri, Imo State, Nigeria. They were sub cultured in nutrient agar at 37^{o}C for 24hrs and stored in the refrigerator before use.

2.4 Antimicrobial activity

The antibacterial activity of the herbal remedies and antibiotics were determined using the well in agar diffusion method [13]. Mueller Hinton agar was poured into petri dish and allowed to set. Wells of 9mm was made in the agar and 25mg/ml of the herbal remedies and 20ug/ml of antibiotics used to fill the wells seeded with test organism respectively. The plates were left to stand for 1hr and then incubated at 37^{o}C for 24hrs. At the end of the incubation period, the appearance of zones of clearing around the wells was checked and determined by measuring the diameter of the zones.

2.5 Combination assay

To determine the combination antimicrobial activity, the bacterial strains were spread evenly on Mueller Hinton agar (MHA) plates [14]. The antibiotic discs were carefully impregnated with 2µL of different herbal medicines and placed on the inoculated plates. The plates were then incubated at 37^{o}C for 24hrs. At the end of the incubation period, the appearance of zones of clearing around the wells was checked and determined by measuring the diameter of the zones. The results were compared with those from the individual antibiotics and herbal medicines.

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2.6 Statistical analysis
The experiments were done in triplicates and the results presented as mean +/- SEM

3. RESULTS AND DISCUSSION

3.1 Antibacterial Activity test
The antibacterial activity of the herbal medicines and antibiotics against the test organisms are presented in the Table 1 and 2 respectively. The activity recorded varied according to the herbal medicine and organism tested. However, the Alomo bitters and Goko cleanser recorded the most antibacterial activity against all test organisms. The diameter zone of inhibition recorded ranged from 12mm to 25mm for Alomo bitters and 11mm to 35mm for Goko cleanser. Yoyo bitters was effective against three organisms with diameter zone of inhibition range of 10mm to 15mm while the least active herbal medicine was Chimanson mighty which was active against one organism only with diameter zone of inhibition of 22mm. Going by the figures recorded, Goko cleanser was the most active and Chimanson mighty the least active.

The different levels of activity demonstrated by the herbal medicines may be attributed to their differences in composition and active ingredients/constituents. Goko cleanser which showed the most activity had the highest number of plant extracts in its formulation and ingredients with 6 plants extracts according to the manufacturers. While the constituents of Chimanson mighty with the least activity was unspecified by the manufacturer. For Alomo and Yoyo bitters, aside the 4 plant extracts they contain also have a high alcohol content which might also have accounted for their activity.

Table 1: Antibacterial Activity of Herbal Medicine

Herbal Remedies	Pseud	E.coli	Staph	Sal	Shi
	Diameter zone of inhibition (mm)				
Alomo Bitters	25	14	17	15	12
Chimanson Mighty	22	-	-	-	-
Goko Cleanser	21	19	11	35	18
Yoyo Bitters	15	10	-	-	14

In relation to the antibiotics tested, chloramphenicol had the most activity against 3 organisms with the highest diameter zone of inhibition 30mm recorded against Staphylococcus aureus. This was followed by tetracycline with activity against 3 organisms and the Ampicillin and Cloxacillin which both had activity against 2 organisms each. *Shigella* sp was resistant to all antibiotics tested while *Salmonella* sp and *Staphylococcus* sp were the most susceptible to 3 out of the 4 antibiotics tested.

Table 2: Antibacterial Activity of Antibiotics

Antibiotics	Pseud	E.coli	Staph	Sal	Shi
	Diameter zone of inhibition (mm)				
Ampicillin	22	-	20	-	-
Cloxacillin	-	13	-	15	-
Chloramphenicol	-	14	30	21	-
Tetracycline	23	-	19	21	-

3.2 Combination activity of the herbal medicine and antibiotics

The two most active herbal medicines Goko cleanser and Alomo bitters were selected for the combination activity testing with all 4 antibiotics and the results shown in Table 3. The activity of Alomo bitters and all four antibiotics showed that the diameter zones of inhibition ranged from 10mm to 35mm. The highest inhibition value (35mm) was recorded for the combination of Alomo bitters and chloramphenicol against *Staphylococcus aureus* while the least value (10mm) was for Alomo bitters and tetracycline against *Shigella* sp. The comparison of the individual medicines and the combination of Alomo bitters with the antibiotics are presented in Figure 1. From the results, the following can be deduced;

i) that the combination of Alomo bitters and Ampicillin showed a positive interaction against *E. coli* and *Staphylococcus aureus* and no interactions (Indifference) against *Pseudomonas*, *Salmonella* and *Shigella* spp.
ii) Alomo and Cloxacillin showed a positive interaction against *E. coli*, a negative interaction against Staphylococcus and Shigella and no interactions (Indifference) against Pseudomonas and Salmonella.
iii) Alomo and Chloramphenicol showed a positive interaction against *E. coli*, Staphylococcus and Salmonella while it showed no interaction (indifference) against Pseudomonas and Shigella.
iv) Alomo and Tetracycline combination showed a positive interaction against Pseudomonas, Staphylococcus and Salmonella, a negative interaction against Shigella and no interaction against *E. coli.*

For Goko cleanser and antibiotics combinations, the antibacterial activity showed that the diameter zones of inhibition ranged from 10mm to 38mm. The highest inhibition value (38mm) was recorded for the combination of Goko cleanser and chloramphenicol against Salmonella while the least value (10mm) was for Goko cleanser and Cloxacillin against *Staphylococcus aureus*. A comparison of the individual medicines and the combination of Goko cleanser with the antibiotics are presented in Figure 2. From the results, the following can be deduced;

i) that the combination of Goko cleanser and Ampicillin showed a positive interaction against Pseudomonas, Staphylococcus and Shigella, negative interactions against Pseudomonas, Salmonella and no interaction (indifference) against *E. coli*.
ii) Goko cleanser and Cloxacillin showed a positive interaction against *E. coli* and Shigella, a negative interaction against Pseudomonas and no interactions (Indifference) against Staphylococcus and Salmonella.

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iii) Goko cleanser and Chloramphenicol showed a positive interaction against all the tested organisms except Shigella which showed no interaction (indifference).

iv) Goko cleanser and Tetracycline combination also showed a positive interaction against all tested organisms except *E. coli* which was indifferent.

From the observations made in this research, there are no known previous reports of the antibacterial activities of these herbal medicines either individually or in combination with antibiotics. This makes this study relevant and a good reference report for subsequent research. However, the active component ingredients of these herbal medicines gotten from extracts of *Aloe-vera, Acinos arventis, Citrus aurantifolis, Chenopodium murale, Cinnamomum aromaticum, Vernonia amydalina, Saccharum officinarum, Allium sativum, Cayanus cajan* and *Zingiber officionale* are known and reported to have antibacterial activities against both Gram positive and Gram negative organisms [15]. Some of these plants extracts such as Allium sativum in combination with some antibiotics have been reported to possess antibacterial synergistic effects against bacterial organisms [12].

Table 3: Combination activity of Herbal Medicine and Antibiotics

Herbs+Antibiotics	Zone of Inhibition (mm)				
	Pseud	E. coli	Staph	Sal	Shi
Alomo + Amp	24	18	25	15	12
Alomo + Clox	25	21	15	15	10
Alomo + Chlo	25	18	35	25	12
Alomo + Tetra	30	14	20	27	10
Goko + Amp	30	19	24	22	20
Goko + Clox	15	22	10	35	21
Goko + Chlo	30	25	36	38	18
Goko + Tetra	31	20	24	36	20

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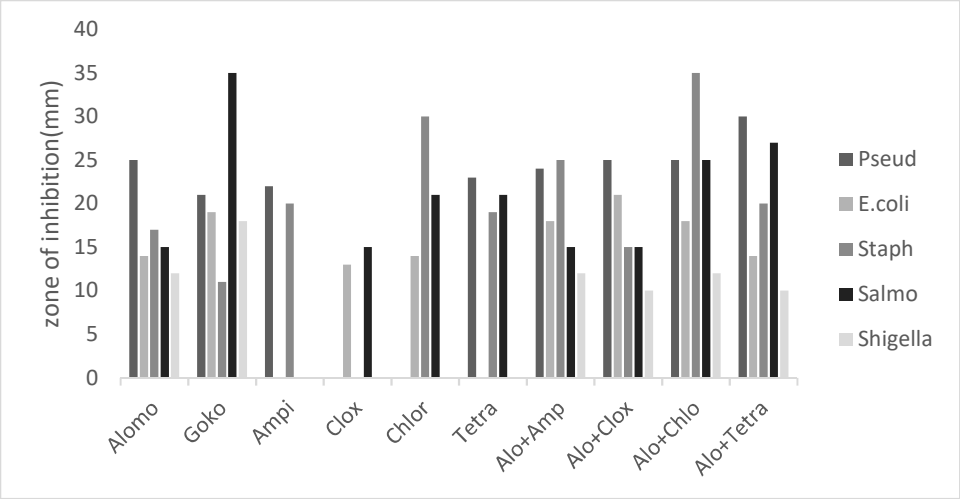

Fig 1: Comparison of the Antibacterial activity of individual test samples and combination of Alomo bitters and Antibiotics

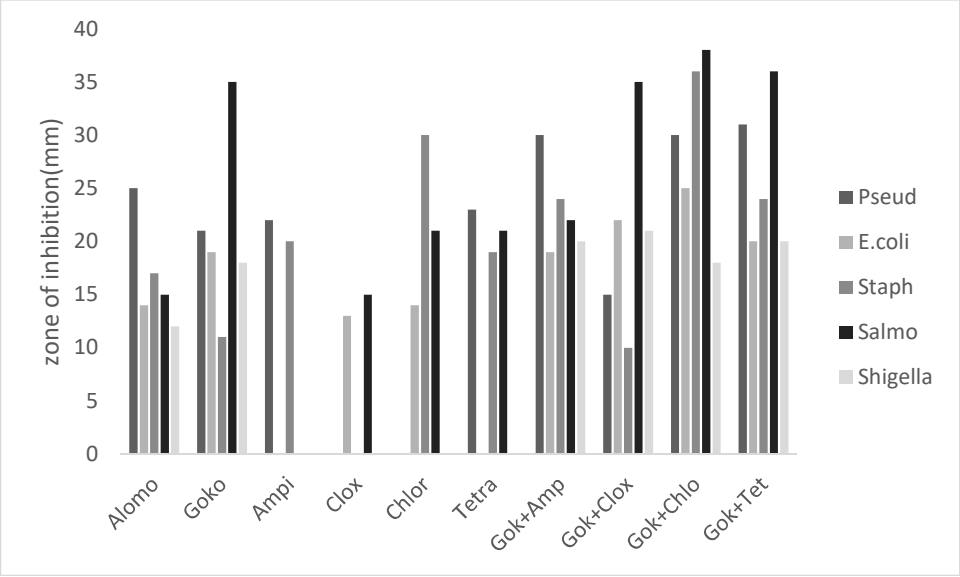

Fig 2: Comparison of the Antibacterial activity of individual test samples and combination of Goko cleanser and Antibiotics

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Therefore, it implies that combination of Alomo bitters and antibiotics gave a 45% positive interaction, 15% negative interaction and 40% non-interaction or indifference. While the combination of Goko cleanser and antibiotics gave a 65% positive interaction, 10% negative interaction and 25 % non-interaction or indifference (Fig 3 and 4).

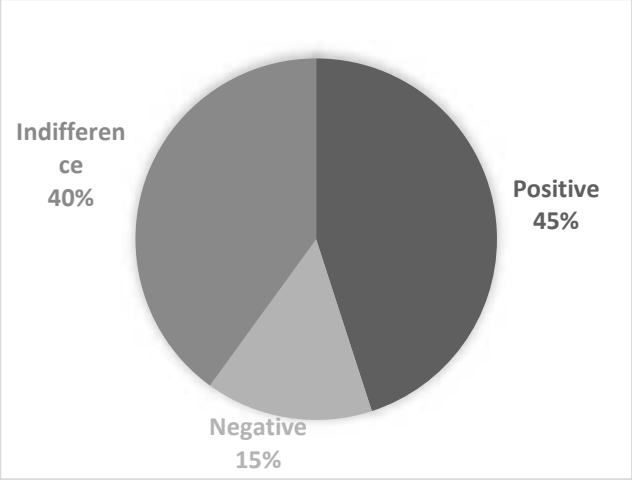

Fig 3: Alomo bitters-Antibiotics drug percentage interaction study

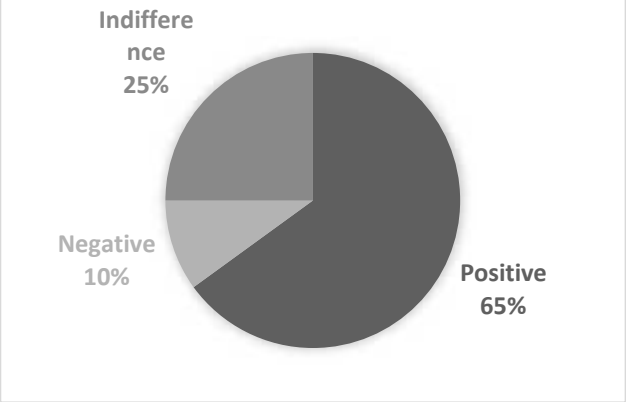

Fig 4: Goko cleanser-Antibiotics drug percentage interaction study

4. CONCLUSION

This indicates that there were more positive interactions which signifies either a synergistic or additive effect from the combinations of the herbal medicine and antibiotics than the negative interactions which signifies an antagonistic effect of the antibiotics-herb combination. While there are still palpable fears in the combined use of antibiotics or other orthodox medicine with herbal medicine in medical practice, this study suggests the possibility of using both kinds of medicine in the treatment and management of microbial infections in a complementary manner.

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