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Research Article

Antibacterial Interaction of Crude Methanol Extract of *Garcinia kola* Seed with Gatifloxacin

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Abstract

Purpose: *Concurrent use of orthodox and herbal medicines is likely to precipitate an overall effect which may or may not be beneficial to the patient. The objective of this study was to evaluate the antimicrobial interaction between the methanol extract of Garcinia kola seed (GKS) which is chewed habitually as a masticatory in many rural communities in Africa and gatifloxacin (GAT), a fourth generation fluoroquinolone.*

Method: *The antimicrobial interaction between these two agents was evaluated by a modification of the checkerboard technique using Bacillus subtilis and Staphylococcus aureus as the test organisms.*

Result: *Results obtained showed that the minimum inhibitory concentration (MIC) of gatifloxacin against both organisms was 1.0 µg/ml while the MICs of the G. kola seed extract were evaluated to be 1.562 mg/ml and 3.125 mg/ml respectively against B. subtilis and S. aureus. Upon combination, synergism was manifested serially against B. subtilis in ratios of 9(GAT):1(GKS) down to 6(GAT) :4(GKS) after which additivity, indifference and antagonism, in that order, were manifested as the ratio of GKS increased in the combination. Against S. aureus, the combined interaction showed a somewhat irregular pattern of effect, including synergism at GAT:GKS ratios of 9:1, 2:8 and 1:9 , antagonism at ratios of 8:2, 5:5 and 4:6 and indifference at GAT:GKS ratios of 7:3, 6:4 and 3:7.*

Conclusion: *The results from this study suggest that the effect of combination of the methanol extract of GKS with gatifloxacin was dependent not only on the ratio of combination but also on the test organism employed for the evaluation. Overall, the combined antimicrobial effect of the interaction between GKS and gatifloxacin was predominantly synergistic against B.subtilis.*

Keywords *Garcinia kola seed, antibacterial interaction, checkerboard technique, Bacillus subtilis, Staphylococcus aureus, gatifloxacin*

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INTRODUCTION

G*arcinia kola* seed (GKS), generally known as 'bitter kola' in Nigeria belongs to a family of tropical plants known as Guttiferae and it grows abundantly throughout west and central Africa[1]. The seed is rich in flavonoids and have equally been shown to have anti-inflammatory and antimicrobial activities; hence it has been employed in the treatment of various ailments[2]. In Nigeria, the seed is chewed for the relief of cough, colds, colic, hoarseness of voice, throat infection and as a masticatory. The plant is also used for the treatment of liver disorder and is used as a chewing stick[3]. The sap from *Garcinia kola* is used for the treatment of parasitic skin diseases while the latex is orally ingested for the treatment of gonorrhea[4]. It is also useful in the eradication of guinea worm infestation[5]. It is equally employed in the management of diabetes on account of its bitter principles[6].

Gatifloxacin is a synthetic broad spectrum fourth-generation 8-methoxy-fluoroquinolone[7]. Gatifloxacin has *in vitro* and *in vivo* activities against a wide range of Gram-negative and Gram-positive aerobic and anaerobic microorganisms[7]. The interest in the present study is being spurred by our observation, over the years, that a large number of people habitually chew *Garcinia kola* and a good number of these people usually continue in this habit unsuspectingly even when they are placed on one kind of drug or the other including antibiotics. Concurrent use of orthodox and herbal medicines is practiced in many urban and rural communities in Africa and Asia including many communities and cities in Nigeria. It is likely that certain interactions may be taking place, without detection, in persons who have this habit of concomitant use of orthodox medicines and herbal drugs. Such interactions may result in either synergistic, antagonistic, indifferent or additive effects.

The aim of this study, therefore, was to investigate, *in vitro,* the therapeutic implications of the common practice of casual chewing of *Garcinia kola* seed as a herbal remedy by patients who may be placed on gatifloxacin for the eradication of one form of bacterial infection or the other and the possible interaction that might result from such a practice.

MATERIALS AND METHODS

Garcinia kola seeds

These were obtained in April, 2006 from people in the Nsukka locality, who cultivate the plant, harvest and market the seeds in their local markets. Authentication of the seeds was done by Mr. A. O. Ozioko of the Bioresources Development and Conservation Programme Center (BDCP), Nsukka, Enugu State, Nigeria and a voucher specimen (PC98032) is preserved in the Pharmacognosy Herbarium, University of Nigeria, Nsukka.

Bacterial media and antibiotic Nutrient agar, Mueller-Hinton Agar (Oxoid, England) and nutrient broth (Merck, Germany) were used as media for the study. Gatifloxacin pure powder (Bristol- Myer Squibb, USA) was used as the synthetic antibiotic.

Reagents

Analytical grades of methanol (Fluka, Germany) and dimethylsulphoxide, DMSO (Merck, Germany) were used for extraction and dilution respectively of the *G. kola* extract.

Test microorganism*s*

Standard cultures of *Escherichia coli* ATCC 9637 and *Staphylococcus aureus* ATCC 13709 were collected from the Bioresources Development and Conservation Program (BDCP) center, Nsukka. Clinical isolates of *Salmonella typhi* was collected from Kenol medical Laboratories, Nsukka while an isolate of *Bacillus subtilis* was obtained from stock cultures in the Pharmaceutical Microbiology laborarory of our University.

Extraction of the active principles of G. kola seed

The *Garcinia kola* seeds were cut into pieces, sun dried for three consecutive days and then pulverized using an end runner mill. The fine powder (360 g) was extracted with one liter of methanol by the cold maceration method for

24 h. The extract was further filtered and allowed to evaporate to a semi-solid residue (23.6 g) which afforded a percentage yield of 6.54.

Maintenance, activation and standardization of stock microbial cultures

The stock microbial cultures were maintained on nutrient agar slants at 4 ^{0}C. In order to

Table 1: The combined antibacterial effect of the methanol extract of *Garcinia kola* seed and gatifloxacin against *Bacillus subtilis*

Drug Combination Ratio (GAT : GKS)	MIC of GAT (µg/ml)	MIC of GKS (µg/ml)	FIC of GAT	FIC of GKS	FIC Index	Activity Index	Effect
10 : 0	0.313	-	-	-	-	-	-
9 : 1	0.141	4.69	0.4499	1.5625×10^{-3}	0.4515	-0.345	Syn
8 : 2	0.250	18.75	0.8	6.25×10^{-3}	0.8063	-0.094	Syn
7 : 3	0.219	28.13	0.7002	9.375×10^{-3}	0.7096	-0.149	Syn
6 : 4	0.094	18.75	0.3002	6.25×10^{-3}	0.3065	-0.5135	Syn
5 : 5	0.313	93.75	1.002	3.125×10^{-3}	1.0333	0.014	Add
4 : 6	0.500	225.0	1.6	7.5×10^{-2}	1.675	0.224	Ind
3 : 7	0.375	262.5	1.2	8.75×10^{-2}	1.2875	0.11	Ind
2 : 8	0.500	600	1.6	2.0×10^{-1}	1.8	0.255	Ind
1 : 9	0.500	1350	1.6	4.5×10^{-1}	2.05	0.312	Ant
0 : 10	-	3000	-	-	-	-	-

***Key** : Syn = Synergism; Ind = Indifference; Add = Additivity; Ant = Antagonism; MICs of GAT and GKS evaluated from agar dilution method against B. subtilis were 1.0 and 1562 µg/ml respectively.*

Table 2: The combined antibacterial effect of the methanol extract of *Garcinia kola* seed and gatifloxacin against *Staphylococcus aureus*

Drug Combination Ratio (GAT : GKS)	MIC of GAT (µg/ml)	MIC of GKS (µg/ml)	FIC of GAT	FIC of GKS	FIC Index	Activity Index	Effect
10 : 0	0.039	-	-	-	-	-	-
9 : 1	0.035	1.172	0.9003	3.9063×10^{-4}	0.9007	-0.045	Syn
8 : 2	0.125	9.375	3.1969	3.125×10^{-3}	3.2	0.505	Ant
7 : 3	0.055	7.031	1.3940	2.3438×10^{-3}	1.3963	0.145	Ind
6 : 4	0.047	9.375	1.1995	3.125×10^{-3}	1.2026	0.080	Ind
5 : 5	0.078	23.438	1.9974	7.8125×10^{-3}	2.005	0.302	Ant
4 : 6	0.125	56.250	3.1969	1.875×10^{-2}	3.2157	0.507	Ant
3 : 7	0.047	32.813	1.1995	1.0938×10^{-2}	1.2104	0.083	Ind
2 : 8	0.016	18.750	0.3997	6.25×10^{-3}	0.406	-0.391	Syn
1 : 9	0.031	84.375	0.8005	2.8125×10^{-2}	0.8286	-0.082	Syn
0 : 10	-	3000	-	-	-	-	-

***Key:** Syn = Synergism; Ind = Indifference; Add = Additivity; Ant = Antagonism; MICS of GAT and GKS evaluated from agar dilution method against S. aureus were 1.0 and 3125.0 µg/ml respectively.*

activate these cultures, subcultures were freshly prepared and incubated at 37 ^{0} C for 18-24 h before use. Standard suspensions of each test microorganism were made by transferring a colony from the subculture into 5 ml of sterile distilled water, and adjusting the volume to obtain a cell population of approximately 1 × 10^6 CFU/ml. A volume of 0.1 ml of such suspensions was used as inoculum in all the tests.

Preliminary antimicrobial screening

Preliminary antimicrobial screening of the *G. kola* seed extract and gatifloxacin was carried out using the cup-plate agar diffusion method[8]. Sterile cork borer having a diameter of 8 mm was used to bore holes into seeded plates containing 20 ml each of solidified Mueller-Hinton agar. A 1 ml volume of each of the two-fold dilution of the extract in DMSO (2.0 mg/ml, 4.0 mg/ml, 8.0 mg/ml and 10.0 mg/ml) was added into each labeled hole using a sterile pipette. The experiment was repeated for all the test microorganisms. Three replicate tests were performed and the plates incubated at 37^{o}C for 24 h. Growth was examined after incubation and the inhibition zone diameter (IZD) around each hole measured. A control experiment was also set up against each test microorganism using DMSO as a control diluent. The whole experiment was similarly repeated for 1 mg/ml of gatifloxacin using sterile distilled water as the solvent for dilution.

Determination of the minimum inhibitory concentration (MIC)

The MIC of the *G. kola* seed extract and gatifloxacin was obtained using the agar dilution technique. A stock solution of the extract (200 mg) was prepared by dissolving 2.0 g of the extract in 10 ml of 50 % DMSO (i.e. one part of DMSO in one part of water). Serial dilutions were made with sterile distilled water to obtain concentrations between 50 mg/ml and 1.5625 mg/ml. A volume of each of the concentrations equal to 0.5 ml was transferred into an agar plate and made up to 20 ml with molten agar and then allowed to set. The surface of the agar was then dried and streaked with the isolates. An over-night (24 h) broth culture was used for this experiment. The same procedure was repeated with gatifloxacin but in this case a stock solution of 4 mg/ml was prepared and the final concentrations obtained in agar plates ranged from 1 mg/ml to 0.00001 mg/ml. Control plates were prepared for both *Garcinia kola* and gatifloxacin. That of *Garcinia kola* had 5 ml of 50 % DMSO in 15 ml of molten agar while that of gatifloxacin had 5 ml of sterile water in 15 ml of molten agar. The plates were then incubated at 37 ^{o}C for 24 h. The MIC was taken to be the lowest concentration which showed no visible growth of each of the test isolate on the agar surface. The experiment in each case was carried out in four replicates.

Evaluation of the interaction between G. kola seed extract and gatifloxacin

Stock solutions of *Garcinia, kola* extract (30mg/ml) and gatifloxacin (0.01mg/ml) were prepared for evaluation of their combined effect on *Staphylococcus aureus* and *Bacillus subtilis*. The concentration range of each antimicrobial agent in combination ranged fron 1/256 times MIC (1/256 × MIC) to 4 × MIC. The two agents were mixed in varying ratios ranging from 0: 10 to 10: 0 of *Garcinia kola* and gatifloxacin in accordance with the continuous variation checkerboard technique. Each of the eleven combinations of these two antimicrobial agents was serially diluted (2-fold) in 3 ml of sterile water into eight places. Two millilitres each of the dilutions of the stock mixtures was seeded into 18 ml of molten agar. After setting, the surface of the agar was then streaked with the test microorganisms. The streaked agar plates were then incubated at 37 ^{o}C for 24 h. The combined effect of the antimicrobials on the test microorganisms was determined and recorded from the fractional inhibitory concentration (FIC) index. The experiment was done in triplicate to ensure reproducibility of results. The FIC index was calculated as follows[9]:

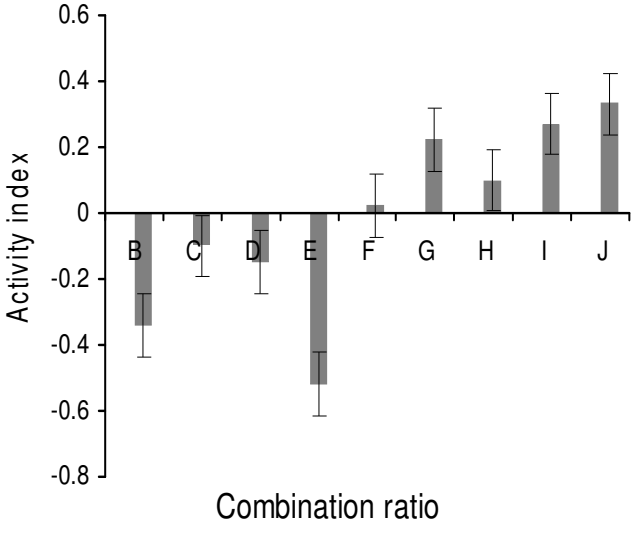

Fig. 1: Activity chart of combinations of methanol extract of Garcinia kola seed and gatifloxacin against Bacillus subtilis. The letters B to J indicate GAT/GKS combination ratio of 9:1, 8:2… 1:9 according to the continuous variation

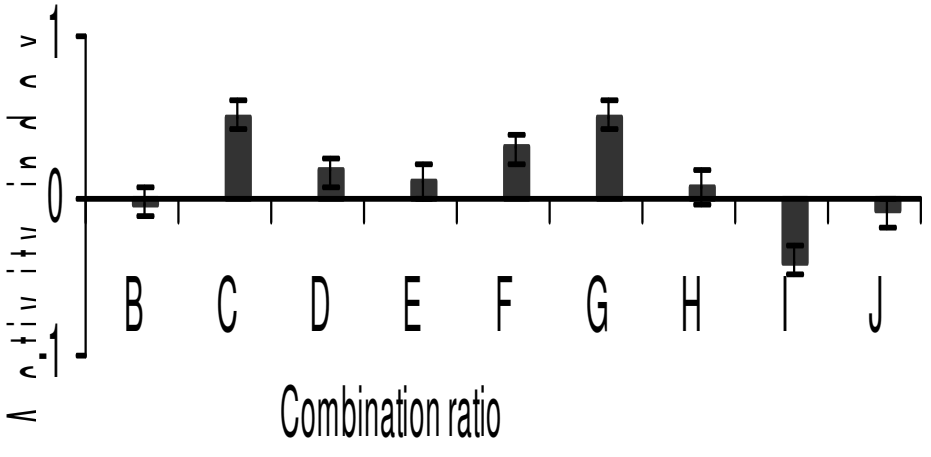

Fig. 2: Activity chart of combinations of methanol extract of Garcinia kola seed and gatifloxacin against Staphylococcus aureus. The letters B to J indicate GAT/GKS combination ratios of 9:1, 8:2, ... 1:9 according to the continuous variation checkerboard

FIC index = $FIC_{GAT} + FIC_{GKS}$ ..... (1)

FIC_{GAT} = Fractional inhibitory concentration of gatifloxacin ..... (2)

FIC_{GKS} = Fractional inhibitory concentration of *G. kola* seed ..... (3)

$$FIC_{GAT} = \frac{\text{MIC of Gatifloxacin in combination with } G.\ kola}{\text{MIC of Gatifloxacin alone}} \quad (4)$$

$$FIC_{GKS} = \frac{\text{MIC of } G.\ kola \text{ seed in combination with Gatifloxacin}}{\text{MIC of } G.\ kola \text{ seed alone}} \quad (5)$$

RESULTS

The MICs of the methanol extract of *G. kola* against *B. subtilis* and *S. aureus* was evaluated to be 1.562 ± 0.015 and 3.125 ± 0.018 mg/ml respectively while that of gatifloxacin was calculated to be 1.0 ± 0.003 µg/ml against both organisms. The recorded MIC values are the mean of four replicate studies. Tables 1 and 2 show the results of the combined antimicrobial effect of the methanol extract of G. kola seed and gatifloxacin against the test microorganisms. The Tables show the combination ratios of the drugs, the MICs of both antimicrobials when acting alone, the FICs of each of the antimicrobials at the different ratios, the FIC index and the activity index. As shown in Table 1, the ratio 6:4 {GAT(6): GKS(4)} exhibited the greatest degree of synergy. Synergism was equally exhibited at GAT/GKS ratios of 9:1, 8:2 and 7:3. Additive effect was manifested at GAT/GKS ratio of 5:5 while an indifferent effect was evident at GAT/GKS ratios of 4:6, 3:7, and 2:8. Antagonism was manifested at GAT/GKS ratio of 1:9.

Table 2 shows the combined activity of methanol extract of *G. kola* seed and gatifloxacin against *S. aureus.* Synergistic effects were recorded at GAT/GKS ratios of 9:1, 2:8 and 1:9. An indifferent effect was exhibited at Gat/GKS ratios of 7:3, 6:4 and 3:7 while antagonism was recorded at GAT/GKS combinations of 8:2, 5:5 and 4:6. Figs. 1 and 2 show the activity charts of combinations of methanol extract of *G. kola* seed and gatifloxacin against *B. subtilis* and *S. aureus* respectively. The bars in the negative axis of the ordinate are those which exhibited synergism while those in the positive axis are those which showed antagonism or additivity. In Fig. 1, B, C, D, and E indicate synergism while F and J depict additivity and antagonism respectively. Indifference is indicated by G, H and I. In Fig. 2, B, I and J indicate synergism; D, E and H show indifferent effects while C, F and G indicate antagonistic effects. The letters B to J indicate GAT/GKS combination ratios of 9:1 down to 1:9 according to the continuous variation checkerboard technique as earlier depicted in Tables 1 and 2.

DISCUSSION

It can be seen from the MIC results that whereas gatifloxacin showed very high activities against *B. subtilis* and *S. aureus* as expected being a broad-spectrum highly active fourth-generation fluoroquinolone, *G. kola* seed extract showed only a marginal activity against the two organisms. The FIC index is interpreted as synergism if its value is less than 1.0, additivity if it is equal to 1.0, indifference if more than 1.0 but less than 2.0 and antagonism if more than 2.0 [9]. It is also clear from Table 1 that the synergy recorded for combinations of gatifloxacin and *G. kola* seed extract decreased as the ratio of *G. kola* seed extract increased in the combination. A probable explanation of this enhanced activity in combination is that the gatifloxacin and the antimicrobial principles in methanol extract of *G. kola* seed may possibly have different mechanisms of action or may be inhibiting two different steps in the same biosynthetic pathway of the organism resulting in an overall synergy at certain combinations. Gatifloxacin is known to act by preventing bacterial replication through inhibition of DNA gyrase while the mechanism of action of *G. kola* seed extract is yet to be completely elucidated[10]. However, it has been noted that two antimicrobial agents may interact antagonistically if one is bacteriostatic and the other is bactericidal[11]. Although the combined antimicrobial effect against *S. aureus,* as can be seen from Table 2, does not show a regular pattern, synergism was recorded at GAT/GKS ratios of 9:1, 2:8 and 1:9. This

could be seen to mean a potentiation of the effect of gatifloxacin against *S. aureus* in the presence of methanol extract of *G. kola* seed. A more critical look at Tables 1 and 2 would reveal that the combined effect of the two antimicrobial agents is not only dependent on the ratio of combination but also on the type of the test microorganism employed as exemplified by *B.subtilis* and *S. aureus*.

A recent study indicated that the ethanolic extract of *G. kola* seed did not affect the activity of some fluoroquinolone antibiotics - norfloxacin and pefloxacin - *in vitro* [12]. It was demonstrated, however, in a recent *in vivo* study that the interaction between an aqueous seed extract of *G. kola* and ciprofloxacin hydrochloride against *Staphylococcus aureus* showed a biphasic effect of an antagonistic effect at an earlier stage and a potentiative (additive) effect at a later stage[10].

CONCLUSION

This study has provided a preliminary evidence of some kind of antibacterial interaction between methanol extract of *Garcinia* kola seed and gatifloxacin against *Bacillus subtilis*. There is an indication that combinations of gatifloxacin and *G. kola* seed extract may have some usefulness in chemotherapy of infections in which *B. subtilis* is implicated especially when the concentration of the extract is kept relatively low. Conversely, the combined effect of the interaction against *S. aureus* may not be highly significant at some ratios of combination of gatifloxacin and the methanol extract of *G. kola* seed. In Nigeria, where *G. kola* seeds are commonly consumed socially and in traditional medicine for the relief of cough and throat infections, the therapeutic implications of consuming the seed concomitantly with gatifloxacin cannot be overlooked.

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