Effect of *Garcinia kola* Seed Extract on the *in-vitro* Anti-bacterial Action of Some Penicillin Analogues

Okore, V. C., Ani, C. C. and Ofokansi, K. C.
Department of Pharmaceutics, University of Nigeria, Nsukka

Corresponding author: Okore, V. C. Department of Pharmaceutics, University of Nigeria, Nsukka

Abstract

A bioactive fraction was obtained from an ethanol extract of the seed of Garcinia kola, Heckel. Its anti-bacterial effect was tested in combination with six penicillin analogues against a broadly sensitive clinical isolate of Escherichia cola. At both inhibitory and sub-inhibitory levels, the extract caused a reduction in the anti-bacterial actions of the penicillins. It is proposed that the antagonistic effect is the result of some form of complexation reaction between the penicillins and flavonoids, saponins or metals present in the seed extract and the penicillins. Garcinia kola seeds are commonly chewed in some parts of Africa either for ethnomedicinal purposes or merely as a masticatory, for pleasure. The results of the interaction between the constituents of the seed and some antibiotics have implications on the therapeutic outcome of such drugs when taken concurrently with Garcinia kola seeds.

Keywords: *Garcinia kola* seed extract, Penicillins, Interactions, Clinical isolates of *E. coli*.

Introduction

The penicillins are among the earliest anti-bacterial agents to be put to clinical use. Although widespread bacterial resistance to the penicillins is now known, they are still the drugs of choice for the treatment of sensitive infections (Turnig, 1995). The widespread use of penicillins especially in many African countries can be attributed to their relative cost-effectiveness (Col and O'Connor, 1987). Coincidentally, in Africa, the use of herbal medicines is also widespread (Iwu, 1993; Farombi, 2003). Experiences reported from various parts of Africa are consistent with the fact that concurrent use of herbal medicines and synthetic drugs are widely practised (Yoder, 1982). The worrisome aspect of this practice is that little attention is given to the consequences of possible interaction of such substances when used concurrently. The absence of such important considerations is more common in situations where the herbal preparations are used not only as medicinal agents but also as habitual or social beverages.

The seed of *Garcinia kola*, Heckel (Fam. Guttiferae) is one such plant material that is widely eaten in Africa for various reasons. The seed is used either as a masticatory or in folk medicine for the treatment of cough, common cold and throat infections. The seed contains a wide variety of bioactive chemicals, such as biflavonoids (Iwu and Igboko, 1982), triterpenes, tannins, saponins, sterols (Braide, 1990), cardiac glycosides and alkaloids (Ebana, *et al.* 1991). Its potential use as a therapeutic agent for a variety of diseases has also been reported (Braide, 1991; Iwu, 1999). Results of a study carried out by Esimone *et al.* (2002) indicate that interactions between *G. kola* seed extracts and certain drugs could occur, which can modify drug action. The purpose of the present study is to investigate the possible interactions of *G. kola* and the penicillins, a group of antibiotics that are readily available, relatively cheap and very commonly prescribed.

Materials and Methods

Bacterial media: Nutrient agar (Oxoid) and nutrient broth (Merck) were used as media for the drug interaction study. McConkey agar (Oxoid) was employed for the isolation of *Escherichia coli*, the bacterium used for this study.

Antibiotics: The penicillin analogues used in the study were: amoxicillin (SmithKlineBeecham, Nigeria), ampicillin (Pharxt Pharma., India), benzylpenicillin (Biochem GmbH, Austria), carbenicillin (SmithKlineBeecham, England), cloxacillin (Hovid Pharma., India) and flucloxacillin (SmithKlineBeecham, Nigeria).

Reagents: Analytical grades of ethyl alcohol (BDH Chemicals, UK) and dimethylformamide, DMF (Merck, Germany) were used for extraction and dilution of the *G. kola* extract respectively.

***Garcinia kola* seeds:** These were obtained from the people in the Nsukka locality, who cultivate the plant and harvest and market the seeds.

Test organism: Clinical isolates were obtained from patients admitted in hospitals in the vicinity of our laboratories. Following isolation on McConkey agar, the organisms, which were suspected to be *E. coli,* were subjected to a variety of biochemical tests, which included indole, methyl-red, Voges-Proskauer, β-galactose, citrate and urease tests. Nine isolates were identified as *E. coli.* In a preliminary test of their sensitivities, the isolates demonstrated varying degrees of sensitivity to the penicillins. Only one isolate (obtained from a diarrhoeal patient!) was sensitive to all the penicillin analogues tested. The study was, therefore, limited to this singular, "naïve", isolate, while the others were preserved as specimens of clinical isolates of *E. coli* in our laboratories. The isolate selected for the study was maintained by weekly sub-culturing

on McConkey agar, incubating at 44 °C for 18 h and storing at 4 °C until the next sub-culturing.

Extraction of active fraction of *G. kola* seed: The husk was removed and the inner seed was grated into a coarse, granular pulp. A mass of 250 g of the pulp was macerated in 500 ml of ethyl alcohol for 24 h with occasional shaking. The ethanol extract was recovered by filtration. The solvent was allowed to evaporate completely at room temperature, and a bi-phasic residue was obtained. This was made up of a clear non-volatile yellowish liquid and a dark-brown solid deposit.

Standardization of bacterial inoculum: Prior to use, the agar culture of the test organism was subcultured on nutrient agar slants and then incubated at 37 °C for 20 – 24 h. The growth was harvested into sterile normal saline solution and the turbidity was adjusted to an optical density of 0.45 at 530 nm. This level of dilution yielded a cell population of approximately 2×10^5 cfu/ml as estimated using the viable cells counting technique.

Determination of dose-response relationship for extract and penicillins: The cup agar plate method was adopted in this determination. Three levels of dilution (×2, ×4, and ×8) were made of the liquid extract using DMF as the diluent. By means of sterile pipettes, two drops of a particular dilution were placed in the cup, made in triplicate, in agar that had been seeded with 0.1 ml of the standardized bacterial suspension. The agar plates were kept at room temperature for 1 h to allow for pre-diffusion of the active principles into the agar, before incubation at 37 °C for 20 – 24 h. In the use of the penicillins, a stock solution of each antibiotic was prepared in DMF and each solution was diluted two-fold serially. Four dilutions were selected such that two drops (approx. 0.4 ml) would provide drug mass of 25, 12.5, 6.3, and 3.1 µg respectively. Thus cups made in the seeded agar were each filled with two drops of the respective antibiotic dilutions. Three replicates of such preparations for each antibiotic were made. The plates were left for 1 h at room temperature allowing for pre-diffusion of the antibiotics before incubation at 37 °C for 20 – 24 h. The mean values of the inhibition zone diameter (IZD) obtained for the extract or penicillins were taken as indices of inhibitory action against the organism. All measurements of IZD were corrected by subtracting, from the mean values, an IZD of 3 mm, which was the value obtained when DMF was used alone under similar experimental conditions. The definite antibiotic masses and the corresponding mean IZDs were used to construct dose-response curves.

Effect of varying ratio combinations of the extract and penicillins: A standardized solution containing 5 µg/ml of the antibiotic was combined in varying volume ratios with the *G. kola* extract. Control runs were also performed in which the extract was replaced with the same volumes of DMF in order to maintain the same antibiotic concentrations. All the agar plates were incubated at 37 °C for 24 h after allowing for pre-diffusion. Inhibition zone diameters from both test and control (after correction) were obtained for comparison.

Effect of sub-inhibitory concentration of extract on penicillin action: Agar medium was prepared to contain the active extract in a dilution of 1:8.5, which was found to bring the extract to just below inhibitory concentration. The agar was seeded with the bacterial suspension and allowed to set. A volume of the penicillin solution yielding 15 µg of drug mass was placed in cups made in the seeded agar plates. For each antibiotic, a control procedure was performed without the *G. kola* extract. In this way it was possible to compare the IZDs produced by the penicillins with or without the influence of the extract.

Results and Discussion

Following the cold maceration of the fresh seeds of *G. kola*, and on complete evaporation of the ethanol solvent, a biphasic extract was obtained. The yellowish, slightly viscous liquid mixed freely with DMF, but precipitated into a turbid colloidal dispersion in water. The liquid extract had inhibitory action against the bacterial isolate and was, therefore, used for the study. About 15 ml of the active fraction was got from the initial mass of 250 g of seed pulp, giving a yield of 6 %. The second portion was a dark-brown resinous matter, which was insoluble in water. When dissolved in DMF, this fraction produced no anti-bacterial action and so it was not further investigated in the present study.

However, some earlier investigators have obtained an ethanol extract of the seed of *G. kola*, and reported that the unfractionated extract had no anti-bacterial effect (Esimone *et al.* 2003). Their finding is in contrast to the results obtained in the current tests. In their procedure, these earlier workers subjected the seeds to harsh atmospheric and extraction conditions. They sun-dried the seeds for three consecutive days and later extracted with the Soxhlet apparatus. Obviously, the material was exposed to substantial amounts of heat and it is probable that much of its bioactive components were destroyed in the process. Care is generally required in handling plant materials to avoid the degradation of the active phytochemical constituents.

Previous investigations on *G. kola* seed have attributed its anti-bacterial effect to the flavonoids (Iwu and Igboko, 1982), which are the major components of an ethanol extract of the seed (Onunkwo *et al.* 2004). Fig. 1 shows that the action of the liquid extract decreased linearly with dilution. From the plots, it was possible to determine the level of dilution at which measurable anti-bacterial action of the extract appeared to be lost. It can be seen that the apparent loss of action was obtained with a dilution of ×8.5. Thus in tests requiring sub-inhibitory levels of the extract, a dilution of at least 8.5 was needed to achieve the desired effect.

Figure 2 is an illustration of the susceptibility of the organism to the test antibiotics. In a study of this nature, it is essential that the test organism is markedly sensitive to the antibiotics under investigation.

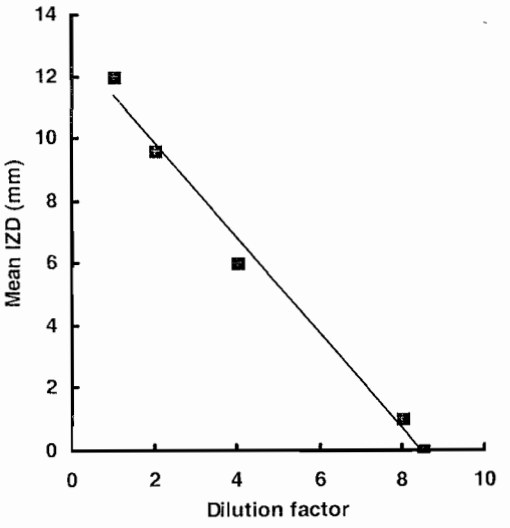


Fig. 1 : Dose-response relation for the *G. kola* extract on *E. coli*

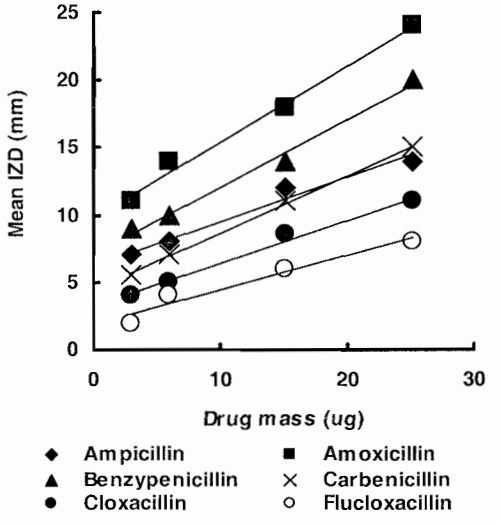


Fig. 2; Mean IZDs representing the susceptibility of *E. coli* isolate to various penicillin analogues

In this way, changes in the qualitative action of the agents can be attributed to interactive effects rather than the influences of microbial susceptibility. It is obvious from the results that the bacterial isolate was appreciably sensitive to all six penicillin analogues used. No other isolate exhibited this level of sensitivity across board.

In combining the *G. kola* extract in varying volume ratios with a 5 µg/ml solution of each of the penicillin analogues, significantly lower values of IZD were obtained than when the antibiotics were used singly, without the extract (Table 1). Flavonoids and saponins have been identified as the dominant phytochemical entities in the ethanol extract of *G. kola* (Onunkwo *et al.* 2004). These secondary metabolites have reactive centres such as α and φ pyrones as well as quinolol moieties that allow for prompt interactions with other functional centres (Iwu and Igboko, 1982). Such interactions with penicillins are possible since the antibiotics possess electron transferring centres and reactive hydrogen atoms that make complexation with flavonoids or saponins possible. Complex formation between such natural organic compounds and penicillins could result in steric hindrance, which would diminish the action of the antibiotics. This may explain why there is only a reduction in action of the penicillins and not total inactivation.

Moreover, elemental analysis of *G. kola* seed has revealed the presence of metals such as iron, copper, calcium and magnesium (Esimone *et al.* 2003). Certain metals are known to interact with some antibiotics resulting in a lowering of potency (Neibergall *et al.* 1966). The activity of some penicillins have been reduced by complexation with copper and other metals (Sher *et al.* 1993). The influence of interacting metallic ions may well constitute an alternative mechanism for the reduction of anti-bacterial action of the penicillins as recorded in this study.

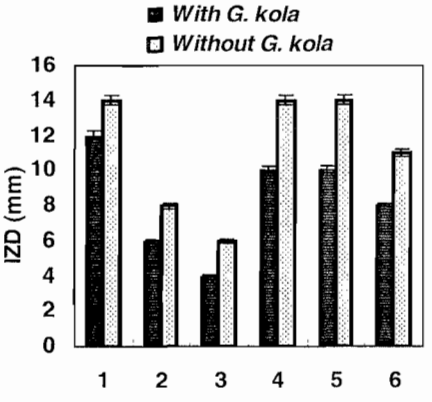
It would appear that the effect of this interaction was largely similar irrespective of whether the *G. kola* extract was combined with the penicillins at inhibitory or sub-inhibitory levels. To verify this assertion, the extract was mixed with nutrient agar in such a manner that a 1:8.5 dilution of the extract was achieved. This level of dilution of the extract was found to correspond to zero IZD. The effect of the sub-inhibitory level of the extract on the action of the penicillins is shown in Fig. 3. There was, generally, a reduction of action in the presence of the extract as compared with the antibiotic acting singly. This observation does not seem to suggest a mode of interference uniquely distinct from the ones proposed earlier in this report. Rather, it goes to affirm the probable therapeutic incompatibility between the penicillins and *G. kola* (which is used ethnomedically for the treatment of oral, throat and upper respiratory tract infections).

Conclusion: This study and similar ones that were reported by Esimone *et al.* (2003) have revealed the rather negative effect of *G. kola* seed extract on the actions of a wide variety of antibiotics. Although these findings have been based entirely on *in vitro* tests, similar effects may be obtained in *in vivo* trials. It might be necessary to determine the level of dilution of the extract at which interference with antibiotic action would be eliminated. Should such level of dilution be comparable to, or more than, the plasma volume of distribution of the active principles following an oral ingestion of *G. kola* seeds, then the speculative concerns expressed about the consumption of *G. kola* seeds while receiving antibiotic therapies would be justified. But based on results so far obtained from empirical tests, there is genuine need for caution in the consumption of *G. kola* seeds by persons who are on antimicrobial chemotherapy.

Table 1: Action of varying ratio combinations of *G. kola* extract and the penicillins.*

Extract-antibiotic ratio	Amoxi-cillin	Ampi-cillin	Benzyl-penicillin	Carbeni-cillin	Cloxa-cillin	Flucloxa-cillin
3:1	4 (27)	10 (17)	3 (25)	11 (16)	10 (16)	12 (11)
2:1	5 (24)	12 (18)	3 (24)	10 (16)	11 (14)	10 (10)
2:3	3 (25)	8 (15)	2 (20	6 (15)	6 (13)	8 (8)
1:1	4 (21)	10 (14)	(20)	8 (15)	10 (10)	9 (8)
3:2	3 (19)	6 (14)	(16)	4 13)	5 (9)	8 (7)

**Data represent the rounded-off means of IZDs (in mm) produced by combinations of extract and the penicillins. (Data in parenthesis are of the antibiotics without the extract).*



Antibiotics with and without *G. kola* effect

(1=Ampicillin; 2=Amoxicillin; 3=Benzylpenicillin; 4=Cloxacillin; 5=Flucloxacillin; 6=Carbenicillin)

Fig. 3: Effect of *G. kola* extract on the activity of various penicillin analogues determined by agar diffusion

References

Braide, V. S. (1991) Antihepatotoxic biochemical effects of kolaviron biflavonoids of *Garcinia kola* seed. *Phytotherapy Res.* 5:35 – 37.

Braide, V. S. (1990) Pharmacological effects of chronic ingestion of *Garcinia kola* seeds in the rat. *Phytotherapy Res.* 4:39 – 41.

Col, N. F. and O'Connor, R. W. (1987) Estimating worldwide current antibiotic usage. *Rev. Inf. Dis.* 9(suppl. 3): S232 – S243.

Ebana, R. U., Madunagu, B. E., Ekpe, E. D. and Otung, I. N. (1991) Microbiological exploitation of cardiac glycosides and alkaloids from *Garcinia kola, Borreria ocymoides, Kola nitida* and *Citrus aurantifolia. J. Appl. Bacteriol.* 71(5): 398 – 401.

Esimone, C. O., Adikwu, M. U., Ndu, O. O., Udeogaranya, P. O., Ezugwu, C. O. and Obonga, W. O. (2003) Effect of *Garcinia kola* seed extract on the antimicrobial properties of some antibiotics *in vitro. J. Pharm. and Allied Sci.* 1(2): 114 – 120.

Esimone, C. O., Nwafor, S. V., Okoli, C. O., Chah, K. F., Uzuegbu, D. B., Chibundu, C., Eche, M. A. and Adikwu, M. U. (2002) *In vivo* evaluation of interaction between aqueous seed extract of *Garcinia kola* and ciprofloxacin hydrochloride. *Am. J. Ther.* 9(4): 275 – 280.

Farombi, E. O. (2003) African indigenous plants with chemotherapeutic potentials and biotechnological approach to the production of bioactive prophylactic agents. *Afr. J. Biotechnol.* 2(2): 662 – 671.

Iwu, M. M. (1999) *Garcinia kola*: a new adaptogen with remarkable immuno-stimulant, anti-infective and anti-inflammatory properties. *Int. Conf. on Ethnomed. and Drug Discovery.* Silver Spring, Maryland, USA. November 3 – 5. Book of Abstracts, p. 26.

Iwu, M. M. (1993) Handbook of African medicinal plants. CRC Press Inc. Boca Raton, USA.

Iwu, M. M. and Igboko, A. O. (1982) Flavonoids of *Garcinia kola* seeds. *J. Nat. Prod.* 45: 650 – 651.

Niebergall, P. J., Hussar, D. A., Grossman, W. A., Sugita, E. T. and Dolussio, J. T. (1966) Metal binding tendencies of various antibiotics. *J. Pharm. Pharmac.* 18: 729 – 736.

Onunkwo, G. C., Egeonu, H. C., Adikwu, M. U., Ojile, J. E. and Olowosulu, A. K. (2004). Some physical properties of tabletted seed of *Garcinia kola. Chem. Pharm. Bull.* 52(6): 649 – 653.

Sher, A., Veber, M., Marott-Gomiscek, M. and Gomiscek, S. (1993) The study of complexation of copper ions with ampicillin II: dynamics of reaction. *Int. J. Pharm.* 99: 112 – 123.

Turnig, J. (1995) Flucloxacillin in infectious diseases (editorial). *Australian Prescriber* 18(3): 54 – 56.

Yoder, S. (1982) Biomedical and ethnomedical practices in rural Zaire: contrasts and complements. *Soc. Sci. and Med.* 16: 1851 – 1857.