African Journal of Biotechnology Vol. 7 (11), pp. 1672-1678, 3 June, 2008
Available online at http://www.academicjournals.org/AJB
ISSN 1684–5315 © 2008 Academic Journals

Full Length Research Paper

In vitro evaluation of the interactions between acetone extracts of *Garcinia kola* seeds and some antibiotics

Sibanda, T. and Okoh, A. I.*

Applied and Environmental Microbiology Research Group (AEMREG), Department of Biochemistry and Microbiology,University of Fort Hare, P/Bag X1314 Alice 5700, South Africa.

Accepted 18 January, 2008

The effect of combinations of the acetone extract of *Garcinia kola* seeds and six first-line antibiotics was investigated by means of fractional inhibitory concentration (FIC) indices as well as by the use of time kill assays. Using the FIC indices, synergistic interactions were observed largely against gram positive organisms (FIC indices of 0.52 - 0.875) with combinations against gram negatives yielding largely antagonistic interactions (FIC indices of 2.0 to 5.0). The time kill assay detected synergy against both gram negative and gram positive organisms with a ≥ 1000 times (≥ $3Log_{10}$) potentiation of the bactericidal activity of tetracycline and chloramphenicol (against *E. coli* ATCC8739 and *K. pneumoniae* ATCC10031) as well as amoxycillin and penicillin G against *Staphylococcus aureus* ATCC 6538. Combinations involving erythromycin and ciprofloxacin consistently gave antagonistic or indifferent interactions. We conclude that the acetone extract of *G. kola* can be a potential source of broad spectrum antibiotics resistance modifying compounds.

Key words: *Garcinia kola*, antibiotic resistance, interactions, resistance modifying compounds.

INTRODUCTION

The wide use of antibiotics in the treatment of bacterial infections has led to the emergence and spread of resistant strains. Infections due to *Staphylococus aureus* are presently resistant to beta-lactams (Cook, 1998), while *Enterococcus* strains are resistant to vancomycin, ampicillin, gentamycin and streptomycin (Montecalvo et al., 1994). Gram negative pathogens such as *Salmonella* species, *Pseudomonas aeruginosa, Klebsiella pneumoniae* have become multi-drug resistant (Fluit et al., 2001). With this emergence of resistance, most old and cheap antibiotics such as the penicillins, the tetracyclines and erythromycin have been rendered ineffective. The loss of clinical efficacy of such previously effective first-line drugs, means that treatment of infections, as a result has to be shifted to second-line or third-line antibiotics that are often more expensive with numerous side effects (Brook et al., 2000). Notwithstanding the fact that new antimicrobial agents are being developed, the past record of resistance development shows that resistant strains often appear a few years after the first clinical use of any antibiotic (Perron et al., 2005).

In the treatment of drug resistant infections, combinations of antibiotics have often been used as this takes advantage of different mechanisms of action. The use of antimicrobial agents displaying synergy is one of the well established indications for combination antimicrobial therapy (Rybak and McGrath, 1996). Antimicrobial synergism occurs when two or more antibiotics, in combination exert an inhibitory effect that is greater than the additive effects of the individual antibiotics. Combinations of antimicrobials that demonstrate an *in vitro* synergism against infecting strains are more likely to result in successful therapeutic outcome. Thus, evidence of *in vitro* synergism could be useful in selecting optimal combinations of antimicrobials for the empirical therapy of serious bacterial infections (Hooton et al., 1984)

Plant extracts and plant derived compounds have long been established to possess antimicrobial activity. However, plant derived compounds have been seen to lack the broad spectrum and potent antimicrobial activity often

*Corresponding author. E-mail: aokoh@ufh.ac.za.

displayed by bacterial or fungal produced antibiotics. Attempts therefore to find potent, nontoxic, broad spectrum antibiotics from plants, have not yielded any good results even though large-scale screens have been undertaken both by pharmaceutical and biotech firms (Lewis and Ausubel, 2006).

It has been hypothesized that, in addition to the production of intrinsic antimicrobial compounds, plants also produce multi-drug resistance (MDR) inhibitors which enhance the activity of the antimicrobial compounds (Stermitz et al., 2000). This hypothesis was tested by Tegos et al. (2002), who showed that the activity of putative plant antimicrobials against gram positive and gram negative organisms was significantly enhanced by synthetic MDR inhibitors of MDR efflux proteins. Those findings provided a basis to believe that plants can be potential sources of natural MDR inhibitors that can potentially improve the performance of antibiotics against resistant strains.

The screening of crude plant extracts for synergistic interaction with antibiotics is expected to provide leads for the isolation of MDR inhibitors. The ability of crude extracts of plants to potentiate the activity of antibiotics has been observed by some researchers and it is anticipated to form the basis for the bioassay directed fractionation of potential resistance modulators from plants. In a study of some Jordanian plants by Darwish et al. (2002), results showed that the efficacy of the antibiotics, gentamycin and chloramphenicol against *S. aureus* were reportedly improved by the use of plant materials. Ahmad and Aqil (2007), also reported that crude extracts of Indian medicinal plants demonstrated synergistic interaction with tetracycline and ciprofloxacin against extended spectrum β-lactamase (ESβL)-producing multidrug-resistant enteric bacteria. Betoni et al. (2006) also observed synergistic interactions between extracts of Brazilian medicinal plants and eight antibiotics on *S. aureus.* The use of *Catha edulis* extracts at sub-inhibitory levels, has been reported to reduce the minimum inhibitory concentration (MIC) values of tetracycline, and penicillin G against resistant oral pathogens, *Streptococcus oralis*, *Streptococcus sanguis* and *Fusobacterium nucleatum* (Al- hebshi et al., 2006).

A number of compounds with an *in vitro* activity of reducing the MICs of antibiotics against resistant organisms have also been isolated from plants. Polyphenols (epicatechin gallate and catechin gallate) have been reported to reverse beta-lactam resistance in Methicillin Resistant *S. aureus* (MRSA) (Stapleton et al., 2004). Diterpenes, triterpenes, alkyl gallates, flavones and pyridines have also been reported to have resistance modulating abilities on various antibiotics against resistant strains of *S. aureus* (Marquez et al., 2005; Smith et al., 2007; Shibata et al., 2005 and Oluwatuyi et al., 2004).

Garcinia kola is a plant that has shown immense potential as a source of chemotherapeutic compounds (Farombi et al., 2002; Han et al., 2005). The seeds of the the plant, commonly known as bitter kola are used in West Africa for the treatment of liver disease, bronchitis, throat infections and in the relief of colic (Iwu et al., 1999). Many phytochemical studies have revealed that the seed is rich in flavonoids and other water soluble polyphenolic compounds (Iwu and Igboko, 1982; Han et al., 2005). While the antibacterial potentials of *G. kola* seed extracts have previously been studied, the interactions between the extracts of this plant and antibiotics have not been documented, especially with regards to its potential as a source of resistance modifying compounds. In this paper, we report the effect of combinations between the acetone extract of *G. kola* seeds and some antibiotics on their antibacterial potencies.

MATERIALS AND METHODS

Plant extract preparation

The extracts of the seed were prepared in accordance to the description of Basri and Fan (2005). One hundred grams of seed powder was steeped in 500 ml of absolute acetone for 24 h with shaking. The resultant extract was centrifuged at 3000 rpm for 5 min at 4°C. The supernatant was then filtered through a Whatman No.1 filter paper while the residue was used for a second extraction with 300 ml of acetone. After the second extraction, the filtrates were concentrated under reduced pressure using a rotary evaporator at 50°C. The concentrated extract was then allowed to dry at room temperature to a constant weight.

Preparation of bacterial inocula

The inocula of the test organisms were prepared using the colony suspension method (EUCAST, 2003). Colonies picked from 24 h old cultures grown on nutrient agar were used to make suspension of the test organisms in saline solution to give an optical density of approximately 0.1 at 600_{nm}. The suspension was then diluted 1:100 by transfer of 0.1 ml of the bacterial suspension to 9.9 ml of sterile nutrient broth before use.

Antibiotics used in this study

The following antibiotics were used in this study: Penicillin G sodium (Duchefa), Amoxycillin (Duchefa), Chloramphenicol (Duchefa), Tetracycline hydrochloride (Duchefa), Erythromycin (Duchefa) and Ciprofloxacin (Fluka).

Determination of the minimum inhibitory concentrations (MIC)

The values for minimum inhibitory concentrations of the antibiotics and plant extracts were determined using the standard method of the European Committee for Antimicrobial Susceptibility Testing (EUCAST, 2000). Dilutions of the antibiotics, ranging from 0.004 – 512 mg l^{-1} in nutrient agar were prepared by incorporating the antibiotic stock solution into molten agar at 50°C. Dilutions of the extract ranging from 0.039 - 20 $mgml^{-1}$ were also prepared by incorporation of the extract in agar at 50°C. After pouring onto plates and allowing the agar to set, the plates were inoculated with standardized innocula of the test bacteria by streaking. Plates were incubated at 37°C for 24 h under aerobic conditions. The MIC was defined as the lowest concentration of the antibiotic or extract that completely inhibited visible growth of the test organism as judged

Table 1. Minimum inhibitory concentrations (MIC) of the antibiotics used.

Test isolate	MIC values (mg l^{-1})					
	Amx	Pen G	Tet	Chlo	Ery	Cip
Staph. aureus ATCC 6538	0.015	0.008	0.25	2	0.25	0.5
Str. faecalis ATCC 29212	0.5	1	8	4	0.5	0.5
Ent. faecalis	0.25	8	32	64	512	0.5
E. coli ATCC 8739	4	32	1	4	128	0.312
K. pneumoniae ATCC 10031	32	64	0.5	1	4	0.015
P. vulgaris CSIR 0030	2	32	16	8	512	0.25

Amx = Amoxycillin; Pen G = Penicillin G; Tet =Tetracycline; Chlo = Chloramphenicol; Ery = Erythromycin; Cip = Ciprofloxacin.

by the naked eye, disregarding a single colony or a thin haze within the area of inoculation (EUCAST, 2000).

Combination studies

The checkerboard method

The study of the combined antimicrobial activity of the plant extracts and antibiotics was done using the agar dilution checkerboard method as described by Mandal et al. (2004). The extract and the antibiotics were combined by incorporation into molten nutrient agar at concentrations ranging from 1/8× MIC to 2× MIC. After setting, the plates were inoculated with standardized cultures by streaking in duplicates. Plates were incubated for 24 h at 37°C after which the MIC values were estimated. The fractional inhibitory concentration (FIC) was derived from the lowest concentration of antibiotic and extract combination permitting no visible growth of the test organisms on the plates (Mandal et al., 2004). The FIC value for each agent was calculated using the formula:

FIC (antibiotic) = MIC of antibiotic in combination / MIC of antibiotic alone

FIC (extract) = MIC of extract in combination / MIC of extract alone

The interactions between the antibiotics and the extracts was assessed in terms of the FIC indices calculated using the formula:

FIC Index = ∑FIC = FIC (antibiotic) + FIC (plant extract)

Combinations were classified as synergistic, if the FIC indices were < 1, additive if the FIC indices were = 1 indifferent if the FIC indices were between 1 and 2 and antagonistic if the FIC indices were >2 (Kamatou et al., 2006). Where more than one combination resulted in a change in the MIC value of the extract or antibiotic, the FIC value was expressed as the average of the individual FIC values as described by Pankey at al. (2005).

The time-kill method

The effect of combinations of the acetone extract of *G. kola* seeds and antibiotics was also evaluated by use of a time-kill assay. This was performed by the broth macrodilution technique following the descriptions of White et al. (1996) and Pankey et al. (2005). The extract and antibiotics were incorporated into 50 ml of nutrient broth at 0.5× MIC and 1× MIC, respectively. Controls consisting of nutrient broth incorporated with the extract and the respective antibiotic alone at the test concentrations included in each experiment.

The test and control flasks were inoculated with each test organism to a final inoculum density of approximately 10^5 cfu ml^{-1}. Immediately after inoculation, aliquots (100 µl) of the negative control flasks were taken, serially diluted in sterile saline and plated on nutrient agar in order to determine the zero hour counts. The test flasks were incubated at 37°C with shaking at 120 rpm. After 24 h of incubation, samples were taken from each test and control flasks, serially diluted in sterile saline and plated (100 µl) on nutrient agar in duplicates. For a better visual observation of the colonies on the agar, 1 ml of 0.5% aqueous solution of 2,3,5 triphenol tetrazolium chloride (Neugebauer and Gilliland, 2005) was added to 100 ml of the molten agar before plating. The plates were incubated at 37°C for 24 h under aerobic conditions. After incubation, the numbers of colonies were enumerated and the mean counts (cfu ml^{-1}) for each test and controls were determined and expressed as $\log_{10}$.

The interactions were considered synergistic if there was a decrease of ≥ 2 $\log_{10}$ cfu ml^{-1} in colony counts after 24 h by the combination compared to the most active single agent (Pankey et al., 2005). Additivity or indifference was described as a < 2 $\log_{10}$ cfu ml^{-1} change in the average viable counts after 24 h for the combination, in comparison with the most active single drug. Antagonism was defined as a ≥ 2 $\log_{10}$ cfu ml^{-1} increase in colony counts after 24 h by the combination compared with that by the most active single agent alone (Pankey et al., 2005; Lee et al., 2006).

RESULTS

The MIC values of the antibiotics used in this study are shown in Table 1. Susceptibility to β-lactam antibiotics, amoxycillin and penicillin G was higher against gram positive organisms (MIC ranges of 0.015 – 0.25 mg l^{-1}) than against gram negatives (MIC ranges of 2 – 32 mg l^{-1}). The macrolide, erythromycin showed the highest MIC values of 128 mg l^{-1} against *E. coli* ATCC 8739 and 512 mg l^{-1} against *P. vulgaris* CSIR 0030 and *Ent. faecalis*. Gram negative organisms showed higher susceptibility to ciprofloxacin (MIC values of 0.015 – 0.25 mg l^{-1}).

The FIC values for the acetone extract, amoxycillin, ciprofloxacin, tetracycline and chloramphenicol are shown in Table 2. The activity of the antibiotics against gram negative organisms was largely reduced by the presence of sub-inhibitory concentrations of the extract. The FIC indices of the antibiotics against gram positive organisms ranged from 0.52 – 1.00 with only *Ent. faecalis* showing an FIC index of 1.625. The activity of all the antibiotics against *K. pneumoniae* ATCC 10031, was reduced due to the presence of the extract. FIC indices for

Table 2. Fractional inhibitory concentration (FIC) values for the combinations between the plant extracts and antibiotics.

Antibiotic	Test isolate	Mean FIC (Antibiotic)	Mean FIC (Extract)	FIC Index	Interaction
Amoxycillin	*Staph. aureus* ATCC 6538	0.196	0.5	0.52	Synergy
	Str. faecalis ATCC 29212	0.5	0.5	1.00	Additivity
	Ent. faecalis	1.25	0.375	1.625	Indifference
Ciprofloxacin	*Str. faecalis* ATCC 29212	0.375	0.25	0.625	Synergy
	Ent. faecalis	0.375	0.5	0.875	Synergy
	E. coli ATCC 8739	2.00	0.06	2.06	Antagonism
	K. pneumoniae ATCC 10031	4.00	0.06	4.06	Antagonism
Chloramphenicol	*Str. faecalis* ATCC 29212	0.375	0.5	0.875	Synergy
	Ent. faecalis	0.234	0.5	0.734	Synergy
	E. coli ATCC 87339	0.5	0.25	0.75	Synergy
	K. pneumoniae ATCC 10031	1.00	1.00	2.00	Antagonism
Tetracycline	*S. faecalis* ATCC 29212	0.375	0.5	0.875	Synergy
	Ent. faecalis	0.3125	0.374	0.686	Synergy
	K. pneumoniae ATCC 10031	4.00	1.00	5.00	Antagonism

Table 3. The determination of synergy between plant extracts and antibiotics using the time kill assay.

Test organism	Changes in bacterial counts (log_{10}cfu/mL) compared with the two agents used alone					
	Amx	Pen G	Chlo	Tet	Ery	Cip
Staph. aureus ATCC 6538	-5.15 (S)	-3.27 (S)	-1.04 (I)	-3.24 (S)	-2.44 (S)	0.00 (I)
Str. faecalis ATCC 29212	-0.88 (I)	0.69 (I)	-1.15 (I)	-1.46 (I)	-2.02 (S)	-2.96 (S)
Ent. faecalis	-1.79 (I)	0.63 (I)	0.003 (I)	-0.37 (I)	-0.21 (I)	0.33 (I)
E. coli ATCC 8739	0.59 (I)	-2.78 (S)	-3.28 (S)	-5.94 (S)	2.73 (A)	4.18 (A)
K. pneumoniae ATCC 10031	1.03 (I)	-0.47 (I)	-3.21(S)	-3.34 (S)	4.78 (A)	5.06 (A)
P. vulgaris CSIR 0030	3.72 (A)	3.54 (A)	2.56 (A)	-0.73 (I)	-0.02 (I)	0.10 (I)

Amx = Amoxycillin; Pen G = Penicillin G; Tet =Tetracycline; Chlo = Chloramphenicol; Ery = Erythromycin; Cip = Ciprofloaxacin (S) = Synergy; (I) = Indifference/Additivity; (A) – Antagonism.

ciprofloxacin, chloramphenicol and tetracycline against *K. pnuemoniae* ATCC 10031 ranged from 2.00 – 5.00.

The time kill effect of combinations between the acetone extract of *G. kola* and antibiotics is shown in Table 3. The extract showed ability to improve the bactericidal effect of beta-lactam antibiotics on gram positive organisms. The bactericidal activity of amoxycillin and penicillin G was increased by 5.15 Log_{10} and 3.27 Log_{10} bases respectively against *Staph. aureus* ATCC 6538. Marginal improvement (less than 2 Log_{10} bases poteniation) in the activity of amoxycillin against *Str. faecalis* ATCC 29212 and *Ent. faecalis* was observed. The bacterial killing activity of protein synthesis inhibitors, tetracycline and chloramphenicol was improved against both gram positive and gram negative organisms with the cidal effect of tetra-cycline showing broad spectrum activity. Erythromycin was strongly potentiated against gram positive organisms *Staph. aureus* ATCC 6538 and *Str. faecalis* ATCC 29212 but the combination was strongly antagonistic against gram negative bacteria, *E. coli* ATCC 8739 and *K. pneumoniae* ATCC 10031.

The nucleic acid inhibitor, ciprofloxacin, showed lack of synergy with the plant extract against all but one of the test organisms (*Str. faecalis* ATCC 29212).

DISCUSSION

The organisms used in this study were reference strains as well as environmental strains of pathogenic organisms often posing problems of drug resistance in clinical settings. In order to assess the effects of combinations between the extracts of the plant and antibiotics, the MIC values of the antibiotics had to be determined as these provide the reference point for defining the interactions. The objective of testing plant extracts for potentials of synergy with antibiotics is to assess if combinations of such extracts with antibiotics can bring about positive changes in the susceptibility of the test strains, thus necessitating the use of strains resistant to the test anti-

biotics. For that reason therefore, the British Society for Antimicrobial Chemotherapy (BSAC) and EUCAST, (2005), recommended MIC breakpoints were used as a way of determining the presence or lack of resistance in the test strains. Although this data is often used in surveillance studies to monitor trends in resistance development, we saw it convenient to apply it in our studies in the absence of a standard.

According to the MIC breakpoints, strains of *Staphylococcus and Streptococcus* with MIC values of ≥ 0.25 mg l^{-1} (for penicillin G), ≥ 2 mg l^{-1} (for amoxicillin), ≥ 2 mg l^{-1} (for tetracycline), ≥ 1 mg l^{-1} (for erythromycin), ≥ 4 mg l^{-1} (for chloramphenicol) and ≥ 1 mg l^{-1} (for ciprofloxacin) are classified as resistant. From our results, *Str. faecalis* ATCC 29212 and *Ent. faecalis* were resistant to penicillin G, tetracycline, chloramphenicol, and erythromycin. The MIC values for these organisms ranged from 4 to 512 times higher than the predicted breakpoint values. The breakpoint values for enteric bacteria are; 16 mg l^{-1} (penicillins), 2 mg l^{-1} (tetracycline), 16 mg l^{-1} (chloramphenicol) and 1 mg l^{-1} (ciprofloxacin) (BSAC and EUCAST, 2005). The enteric bacteria used in this study showed varying levels of susceptibity to the test antibiotics. *K. pneumoniae* ATCC 10031 showed reduced susceptibility to both penicillin G and amoxycillin while *E. coli* ATCC 8739 and *P. vulgaris* CSIR 0030 were more susceptible to amoxycillin. The enteric organisms were generally susceptible to chloramphenicol and ciprofloxacin but showed high MIC values against erythromycin. The presence of such elevated MIC values of some of the organisms used in this study against common front-line antibiotics reflects the common presence of resistance mechanisms universally present in bacteria, and justifies the need to seek strategies to inhibit such mechanisms.

Combinations of antibiotics and the acetone extract of *G. kola* seeds were investigated for possible synergistic interactions. In the checkerboard method, synergy is based on the increased susceptibility of the test organism to the presence of both antimicrobial agents which is reflected by changes in the MIC values (Odds, 2003). Synergy between the plant extract and antibiotics using the FIC indices was detected mainly against gram positive organisms. The synergy was detected for combinations involving amoxycillin, ciprofloxacin, chloramphenicol and tetracycline. Since synergy was not specific to any class of antibiotics, it is likely that the target for this interaction could be the cell membrane since it is the fundamental difference between gram negative and gram positive organisms. There is need therefore, to establish the molecular basis of this interaction. The synergy against *Str. faecalis* ATCC 29212 and *Ent. faecalis* is significant as these organisms were resistant to penicillin G, tetracycline, chloramphenicol, and erythromycin with MIC values much higher than their predicted breakpoints. Although the level of antibiotic potentiation was low (FIC indices of 0.52 - 1.00) as not to lead to a restoration of susceptibility (lowering the MIC values to below the breakpoint values) the results seem promising considering that crude extracts were used. The potentiation is likely to have been much more pronounced if pure compounds were used.

As an alternative method, the time kill assay was also used to assess the effect of combinations of the extracts of *G. kola* seeds and antibiotics. This method was based on a comparison of the killing rate of the combination to that of the individual agents. In the experiment, the extract was incorporated at sub-inhibitory concentrations (1/2× MIC) with the antibiotic at the minimum inhibitory concentration.

In contrast to the checkerboard method, the time kill assay detected synergy against both gram positive and gram negative organisms. Strong synergistic interactions with the extract were observed in combinations involving beta-lactams (amoxycillin and penicillin G) as well as protein synthesis inhibitors, tetracycline and erythromycin against *Straph. aureus* ATCC 6538. Combinations involving tetracycline and chloramphenicol were highly bactericidal against *E. coli* ATCC 87339 and *K. pneumoniae* ATCC 10031 with a more than 1000 fold (> 3 Log_{10}) potentiation of the antibiotic (Table 3). Combinations involving erythromycin and ciprofloxacin against the same gram negative organisms were largely antagonistic. The synergy detected in this study was not specific to any group of organisms or class of antibiotics. This suggests that crude extracts of this plant could be containing a mixture of compounds that can enhance the activity of different antibiotics. The seeds of *G. kola* have been known to contain a number of antimicrobial compounds (Iwu et al., 1999) such as polyphenols and flavonoids. The antimicrobial and resistance modifying potentials of naturally occurring flavonoids and polyphenolic compounds have been reported in other studies such as Cushnie and Lamb, (2005), Sato et al., (2004). This would suggest that, the synergy with antibiotics observed in this study could be attributable to such compounds. Some of these compounds like polyphenols have been shown to exert their antibacterial action through membrane perturbations. This perturbation of the cell membrane coupled with the action of beta-lactams on the transpeptidation of the cell membrane could lead to an enhanced antimicrobial effect of the combination (Esimone et al., 2006). It has also been shown that some plant derived compounds can improve the *in vitro* activity of some peptidoglycan inhibiting antibiotics by directly attacking the same site (i.e. peptidoglycan) in the cell wall (Zhao et al., 2001).

While the above explanations may account for the synergy between the extracts and beta-lactam antibiotics that act on the cell wall, it might not apply in the case of the observed synergy with other classes of antibiotics with different targets such as tetracyclines, erythromycin, ciprofloxacin and chloramphenicol. Bacterial efflux pumps are responsible for a significant level of resistance to antibiotics in pathogenic bacteria (Kumar and Schweizer, 2005). Some plant derived compounds have been observed to enhance the activity of antimicrobial com-

pounds by inhibiting MDR efflux systems in bacteria (Tegos et al., 2002). 5'-methoxyhydnocarpin is an example of an inhibitor of the NorA efflux pump of *S. aureus* isolated from *Berberis fremontii* (Stermitz et al., 2000). It is likely that the acetone extract of *G. kola* seeds could be containing potential efflux pump inhibitors. Such compounds are likely to be broad spectrum efflux inhibitors considering that the synergistic effect of the extract was observed on both gram positive and gram negative organisms as well as in combination with, cell wall inhibiting and protein synthesis inhibiting antibiotics. In fact, some broad spec-trum efflux pump inhibitors have been isolated from some plants. Smith et al. (2007) reported one efflux inhibitor (ferruginol) from the cones of *Chamaecyparis lawso-niana*, that inhibited the activity of the quinolone resistance pump (NorA), the tetracycline resistance pump, (TetK) and the erythromycin resistance pump, (MsrA) in *S. aureus*.

The strong synergy observed between the extracts of *G. kola* is a significant finding demonstrating the therapeutic potentials of this plant.

Conclusion

The extracts of *G. kola* seeds showed potentials of synergy in combination with some antibiotics against reference strains of pathogenic organisms often presenting with problems of drug resistance. The detection of synergy between crude extract of *G. kola* and antibiotics demonstrates the potential of this plant as a source of antibiotic resistance modifying compounds. It is necessary to carry out a bioassay guided fractionation of the acetone extract of this plant in a bid to isolate and identify the compounds responsible for the synergistic activity with antibiotics. An elucidation of the mechanisms of action of these compounds must be followed by toxicity and *in vivo* tests to determine the therapeutic applicability of such compounds in combination therapy. These are subjects of on-going investigation in our research group.

ACKNOWLEDGEMENT

The authors are grateful to the National Research Foundation (NRF) of the Republic of South Africa for financial support.

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