academicJournals

Vol. 8(28), pp. 1360-1367, 25 July, 2013
DOI 10.5897/SRE2013.5515
ISSN 1992-2248 © 2013 Academic Journals
http://www.academicjournals.org/SRE

Scientific Research and Essays

Full Length Research Paper

Interaction of the extracts of three medicinal plants with antibiotics against some antibiotic resistant bacteria

Eze, E. A.*, Oruche, N. E. and Eze, C. N.

Department of Microbiology, University of Nigeria, Nsukka, Nigeria.

Accepted 4 July, 2013

The outcome of *in vitro* interaction of standard antibiotics with ethanolic leaf extracts of three medicinal plants (*Picralima nitida, Chromolaena odorata* and *Aspilia africana*) against antibiotic resistant bacteria was investigated by agar disc diffusion methods and macrobroth dilution techniques. At least three isolates each of *Escherichia coli, Staphylococcus aureus* and *Pseudomonas aeruginosa* that were resistant to more than two of the following antibiotic discs were used: erythromycin, chloramphenicol, tetracycline, norfloxacin and ciprofloxacin. A subinhibitory concentration (1/4th MIC) of the plant extracts were combined with the antibiotics against the resistant bacteria isolates using both antibiotic disc diffusion technique and macrobroth dilution test. The extract of *A. africana* enhanced the activities of the test antibiotics (except erythromycin) against resistant *E.* coli, and also enhanced the activities of ciprofloxacin, norfloxacin and chloramphenicol against antibiotic resistant *P. aeruginosa*. It also enhanced the activities of tetracycline and norfloxacin against antibiotic resistant *S. aureus.* The extract of *C. odorata* greatly enhanced the activities of the antibiotics (except erythromycin) against the resistant *P. aeruginosa* and also potentiated the activities of chloramphenicol and tetracycline against resistant *S. aureus*. Although the extract of *P. nitida* potentiated few antibiotics such as tetracycline and chloramphenicol against resistant *E. coli*, the extract exhibited high levels of antagonism with ciprofloxacin and norfloxacin against almost all the test bacteria. These results suggest that extracts of *C. odorata* and *A. africana* could be good sources of multidrug resistance inhibitors, and indicate that indiscriminate co-administration of antibiotics with some herbal drugs such as those from *P. nitida* could be therapeutically wasteful. Their use in combination with conventional antibiotics should be further studied for *in vivo* activities. This may lead to the development of much needed drug enhancing preparations.

Key words: Medicinal plants, antibiotics, antibiotic-extract combination, multidrug-resistant bacteria.

INTRODUCTION

The emergence and spread of multidrug resistance as a phenomenon among bacterial pathogens has been a major problem confronting the field of antibacterial chemotherapy in the recent times. However, it has been found that, in addition to the production of intrinsic antimicrobial compounds (Stefanovic and Comic, 2012; Bama et al., 2012), some medicinal plants also produce multidrug resistance inhibitors which enhance the activities of antibiotics against multidrug resistant bacteria pathogens (Stermitz et al., 2000). It is this finding that prompted efforts in screening of crude extracts for synergistic interaction with standard antibiotics against resistant bacteria as this would pave the way for possible isolation of multidrug resistance inhibitors of plant origin. A study of this type would also help to indicate the class of antibiotics that could be rightly combined with certain

*Corresponding author. E-mail: akachieze@yahoo.com. Tel: +2348066371727.

herbal drugs in ethnomedicine against certain infections.

A number of synthetic multidrug resistance (MDR) inhibitors have been developed, but none has found clinical application due to their toxic properties (Stavri et al., 2007). This makes the screening of medicinal plant extracts an inevitable alternative in the search for resistance modifying agents.

In this study, crude ethanolic leaf extracts of three medicinal plants (*Picralima nitida, Aspilia africana* and *Chromolaena odorata*) were investigated for their ability to potentiate the activities of some antibiotics against antibiotic resistant bacteria including the resistant isolates of *Staphylococcus aureus, Escherichia coli* and *Pseudomonas aeruginosa*. The test antibiotics include: ciprofloxacin, norfloxacin, tetracycline, chloramphenicol and erythromycin.

A. africana and *C. odorata* are plants used in folklore medicine for topical treatment and control of wound infections and skin diseases in some parts of eastern Nigeria, while *P. nitida* is administered orally for treatment of various diseases including malaria and typhoid fever (Kayode et al., 2007; Nkere and Iroegbu, 2005; Schmidt and Schilling, 2000).

MATERIALS AND METHODS

Plant materials

The plants used for this study (*P. nitida, C. odorata and A. africana*) were collected from Ozubulu in Anambra State, in Eastern Nigera. The plants were identified by A.O. Ozioko, a former plant taxonomist at the herbarium section of the Department of Botany, University of Nigeria, Nsukka.

Extraction of active ingredients

The leaves of the plants were plucked, rinsed with water and air-dried at room temperature for several days. The dried leaves were pulverized using a milling machine to obtain fine powder. The active ingredients were extracted by percolation method using 95% ethanol. Briefly, 100 g of each leaf powder was added to 900 ml of 95% ethanol. The mixture was covered, and shaken every 30 min for 6 h, and then allowed to stand for 48 h for extraction. The mixture was then separated by passing through Whatman's No. 1 filter paper, after which the filtrate containing the active ingredients was evaporated to dryness under air pressure. The dried crude extracts were aseptically stored in the refrigerator at 4°C for the study.

Screening for the antibiotic resistance among the test bacteria

The isolates of *E. coli, S. aureus* and *P. aeruginosa* were screened for antibiotic resistance using Kirby-Baurer disc diffusion technique (Cheesbrough, 2000). The following antibiotic discs were used: ciprofloxacin (5 µg), norfloxacin (10 µg), chloramphenicol (30 µg), tetracycline (30 µg) and erythromycin (10 µg). Resistant isolates were chosen based on the interpretive criteria recommended by the Clinical and Laboratory Standard Institute (CLSI, 2011). At least three resistant isolates each of the test organisms (*E. coli, S. aureus* and *P. aeruginosa)* that were resistant to more than two of the above antibiotic discs were selected for the study.

Determination of minimum inhibitory concentration of the crude plant extracts on the antibiotic resistant isolates

The MICs of the extracts were determined by macrobroth dilution technique following the recommendations of the Clinical and Laboratory Standard Institute (CLSI, 2006). Different concentrations of the extract ranging from 50 to 0.78125 mg/ml were prepared in tubes of Muellar Hinton broth by two-fold serial dilutions. Then 1 ml of an overnight nutrient broth culture of the drug resistant bacteria isolates (adjusted to 0.5 MacFarland turbidity standards) was added to each tube. The tubes were incubated at 37°C for 24 h. The experiment was conducted in duplicate. Control tubes were also set up including a tube of Mueller Hinton broth containing the test organism without extract and then the tubes containing different concentrations of the extract in the broth without the test organism. The concentration in the first tube in the series with no visible growth after 24 h incubation period was taken as the MIC of the extract. One-quarter (¼) of this MIC value was used as the sub-inhibitory concentration of the extract in antibiotic resistance modulation experiment.

Antibiotic resistance modulation assay by disc diffusion

The resistance modifying potency of the plant extracts was determined by combining the sub inhibitory concentration of the extracts ($1/4^{th}$ MIC) with the antibiotic discs against the resistant bacteria (Bama et al., 2012; Gibbons et al., 2003). An appropriate dilution of plant extracts in 10% DMSO was incorporated into a specific volume of molten Mueller Hinton agar at 50°C to achieve the final extract concentration equivalent to ¼th MIC that has been predetermined for that particular resistant bacterial organism. The media was then poured into Petri plates and allowed to solidify. The plates were then inoculated with a standardized 18 h old broth culture of the test bacteria isolates by using a sterile cotton tipped swab. The activities of the antibiotics on the test organism were then evaluated by Kirby-Baurer disc diffusion techniques using the same antibiotic discs prepared in the laboratory. The plates without the plant extracts were also inoculated and tested as controls. The experiment was carried out in duplicates.

The inhibition zone diameter of each antibiotic disc was measured after incubation at 37°C for 24 h. The effects of the plant extract on the activity of the antibiotic discs against the resistant organisms was evaluated by comparing the size of inhibition zone diameters in plates containing plant extracts and in control plates without plant extract.

Evaluation of resistance modifying activity of the plant extracts by macrobroth dilution test

The resistance modifying potency of the plant extracts was also evaluated using macrobroth dilution technique by determining the minimum inhibitory concentration (MIC) of the antibiotics on the resistant bacteria in the presence and in absence of sub-inhibitory concentration ($1/4^{th}$ MIC) of the plant extract (Mahamoud and Cheralier, 2007; Gibbons et al., 2003; Bama et al., 2012).

The MICs of the antibiotics were determined by macrobroth dilution method following the standard procedures of the Clinical and Laboratory Standards Institute (CLSI, 2011). The antibiotic was reconstituted in appropriate solvent and different ranges of two-fold serial dilutions were made from these stock solutions in tubes of I ml sterile Mueller Hinton broths. Depending on the value of the predetermined MIC of each crude extract on a particular antibiotic resistant organism, about 200 to 300 µL of a known concentration

Table 1. Resistance profile of the antibiotic resistant bacterial isolates.

Organism	Antibiotic discs and their mean IZD (mm)				
	Cip	Nor	Ch	Tet	Ery
E. coli	14 (R)	19 (S)	- (R)	- (R)	- (R)
P. aeruginosa	13 (R)	13 (I)	- (R)	- (R)	- (R)
S. aureus	22 (S)	15 (I)	10 (R)	- (R)	- (R)

Cip = Ciprofloxacin (5 µg); Nor = norfloxacin (10 µg); Ch = chloramphenicol (30 µg); Tet = tetracycline (30 µg); Ery = erythromycin (10 µg); (S) = susceptible; (I) = intermediate; (R) = resistant; (-) = no zone of inhibition; IZD=inhibition zone diameter.

Table 2. The average MIC values of the plant extracts on the antibiotic-resistant bacteria.

Plant extract	Mean MIC (mg/ml)					
	E. coli		*S. aureus*		*P. aeruginosa*	
	MIC	1/4th MIC	MIC	1/4th MIC	MIC	1/4th MIC
P. nitida	2.340	0.5856	2.340	0.5856	4.69	1.172
C. odorata	5.470	1.367	1.95	0.4870	6.25	1.56
A. africana	2.730	0.6834	2.340	0.5856	3.906	0.9766

MIC = Minimum inhibitory concentration.

of the extract is added separately to 1 ml tubes of Mueller Hinton broth containing the serially diluted antibiotic to obtain a final extract concentration equal to ¼th MIC of that extract. Then 1 ml of an overnight broth culture of the test resistant organism adjusted to 10^6 CFU/ml was added to each tube of serially diluted antibiotics in 1 ml of Mueller Hinton broth (the tubes which also contain ¼th MIC of the plant extract). Tubes of 1 ml Mueller Hinton broth containing the serially diluted antibiotics without the plant extract were also inoculated with the test resistant organism. The experiment was conducted in duplicate and incubated at 37°C for 24 h. The following served as controls: (i) a tube of 1 ml Mueller Hinton broth containing the test organism without antibiotic and without plant extract. (ii) a blank tube of 1 ml Mueller Hinton broth without any antibiotic, plant extract or test organism.

At the end of the incubation, the tubes were examined for any difference in the MIC of the antibiotic containing sub-inhibitory concentration of crude extract and the MIC of the antibiotic containing no extract.

Statistical analysis

One way Analysis of Variance (ANOVA) test was used to determine if there was any significant ($p \leq 0.05$) difference in the potentiation and/or antagonistic effects of the test extracts on the antibiotics against the test organisms. The difference in IZD values between the effects of the extract on one drug against a particular isolate and another drug on the same isolate was used as values in the ANOVA test.

RESULTS

Table 1 shows the resistance profile of the antibiotic resistant bacteria that were screened by antibiotic disc diffusion test. The MICs of the crude plant extracts on the antibiotic resistant organisms with their corresponding ¼th concentrations used in the antibiotic-extract combination assay were presented in Table 2.

An extract is considered to exhibit synergy with antibiotic if the combinations of the antibiotic with the plant extract (¼ MIC) lead to up to 5 mm increase in inhibition zone diameter of the discs or if such combination leads to up to 2-fold decrease in the MIC of the antibiotic (Schmitz et al., 1998; Ahmad and Aqil, 2007; Adwan and Mhanma, 2008). Tables 3 and 4 show the effects of interaction of extract of *C. odorata* (¼ MIC) with the test antibiotics against the resistant bacteria in agar-disc diffusion test and in macrobroth dilution experiment respectively. The extract enhanced the activities of all the test antibiotics (except erythromycin) against resistant isolates of *P. aeruginosa* as evidenced by the increase in the inhibition zone diameter of the antibiotic discs and up to four-fold reductions in the MICs of the antibiotics. Other cases of enhancement were also observed with some antibiotics against two or more isolates of the resistant organisms. The *C. odorata* extract had a more significant ($p \leq 0.05$) synergistic effect on tetracyclines against *S. aureus* when compared with both ciprofloxacin (Cip) and chloramphenicol (Ch). The difference was also significant between Cip and Ch against *E. coli* with a more profound synergistic effect on Ch.

Table 5 shows the effects of the extract of *A. africana* on the activities of the antibiotic against the resistant bacteria in the antibiotic disc diffusion test, while Table 6 shows the effects of the extract on the MICs of the antibiotics against the resistant bacteria in macrobroth

Table 3. Effects of extract of *C. odorata* on the activities of antibiotic discs against antibiotic-resistant bacterial isolates.

Organism	Number of isolates	Antibiotic disc	Mean IZD (mm)		Remarks
			Antibiotic disc alone	Antibiotic discs + Extract (1/4th MIC)	
E. coli	3	Cip	14	16	No effect (in 2 isolates)
		Nor	19	20	No effect (in 2 isolates)
		Tet	-	8	Synergism (in 2 isolates)
		Ery	-	-	No effect (in all isolates)
		Ch	-	11	Synergism (in all isolates)
P. aeruginosa	4	Cip	12	20	Synergism (in all isolates)
		Nor	15	22	Synergism (in 3 isolates)
		Tet	-	9	Synergism (in 3 isolates)
		Ery	-	-	No effect (in all isolates)
		Ch	-	11	Synergism (in 2 isolates)
S. aureus	3	Cip	23	30	Synergism (in all isolates)
		Nor	15	24	Synergism (in all isolates)
		Tet	-	12	Synergism (in 2 isolates)
		Ery	-	-	No effect (in all isolates)
		Ch	8	12	Synergism (in all isolates)

Cip = Ciprofloxacin (5 µg); Nor = norfloxacin (10 µg); Ch = chloramphenicol (30 µg); Tet = tetracycline (30 µg); Ery = erythromycin (10 µg); (-) = no zone of inhibition.

Table 4. Effects of *C. odorata* extract on the activities of the antibiotics against antibiotic resistant bacteria in macrobroth dilution test.

Organism	Number of isolates	Antibiotic	Mean MIC (µg/ml)		Remarks
			Antibiotic alone	Antibiotic + Extract (1/4th MIC)	
E. coli	3	Cip	8	8	No effect (in all isolates)
		Nor	6	6	No effect (in 2 isolates)
		Tet	32	32	No effect (in 2 isolates)
		Ery	ND	ND	-
		Ch	> 128	32	Synergism (in 2 isolates)
P. aeruginosa	4	Cip	26.7	6	Synergism (in all isolates)
		Nor	3.3	0.42	Synergism (in 2 isolates)
		Tet	> 128	42.67	Synergism (in 3 isolates)
		Ery	ND	ND	-
		Ch	> 128	64	Synergism (in all isolates)
S. aureus	3	Cip	< 0.125	<0.125	-
		Nor	4	4	No effect (in all isolates)
		Tet	28	4	Synergism (in all isolates)
		Ery	170.67	213.33	Antagonism (in all isolates)
		Ch	> 128	32	Synergism (in all isolates).

Cip = Ciprofloxacin (5 µg); Nor = norfloxacin (10 µg); Ch = chloramphenicol (30 µg); Tet = tetracycline (30 µg); Ery = erythromycin (10 µg). (-) = no zone of inhibitions. ND = not determined. MIC = minimum inhibitory diameter.

dilution tests. Again, although the combination was not uniformly synergistic, the extract of *A. africana* enhanced the activities of the antibiotics against some of the isolates of the resistant organisms as shown by the increase in the inhibition zone diameter of the antibiotic discs and decrease in the MIC of the antibiotics.

Table 5. Effects of extract of *A. africana* on the activities of antibiotic disc against antibiotic- resistant bacteria**.**

Organism	Number of isolates	Antibiotic	Mean IZD (mm)		Remarks
			Antibiotic disc alone	Antibiotic disc + Extract (1/4th MIC)	
E. coli	3	Cip	16	20	Synergism(in all isolates)
		Nor	21	30	Synergism (in all isolates
		Tet	-	11	Synergism (in 2 isolates
		Ery	-	-	No effect (in all isolates)
		Ch	-	-	No effect (in 2 isolates)
P. aeruginosa	4	Cip	11	19	Synergism (in 3 isolates)
		Nor	13	20	Synergism (in 2 isolates)
		Tet	-	11	Synergism (in all isolates)
		Ery -	-	-	No effect (in all isolates)
		Ch	-	9	Synergism (in all isolates)
S. aureus	3	Cip	24	30	Synergism (in 2 isolates)
		Nor	14	22	Synergism (in all isolates)
		Tet	-	11	Synergism (in all isolates)
		Ery	-	10	Synergism (in 2 isolates)
		Ch	12	11	No effect (in all isolates)

Cip = Ciprofloxacin (5 µg); Nor = norfloxacin (10 µg); Ch = chloramphenicol (30 µg); Tet = tetracycline (30 µg); Ery= erythromycin (10 µg). (-) = no zone of inhibition. IZD = inhibition zone diameter.

Table 6. Effects of *A. africana* extract on the activities of antibiotics against antibiotic resistant bacteria in macrobroth dilution test.

Organism	Number of isolates	Antibiotic	Mean MIC (µg/ml)		Remarks
			Antibiotic alone	Antibiotic + Extract (1/4th MIC)	
E. coli	3	Cip	10.67	2	Synergism (in all isolates)
		Nor	12	2	Synergism (in 2 isolates)
		Tet	32	3.3	Synergism(in all isolates)
		Ery	ND	ND	-
		Ch	> 128	96	Synergism (in 2 isolates)
P. aeruginosa	4	Cip	24	3	Synergism (in 2 isolates)
		Nor	3.3	0.67	Synergism(in 3 isolates)
		Tet	213.3	213.3	No effect (in 3 isolates)
		Ery	ND	ND	-
		Ch	> 128	64	Synergism (in 2 isolates)
S. aureus	3	Cip	<0.125	<0.125	-No effect (in all isolates)
		Nor	4	0.5	Synergism(in all isolates)
		Tet	32	4	Synergism (in all isolates)
		Nor	106.7	106.7	No effect(in all isolates)
		Ch	128	128	No effect (in 2 isolates)

Cip = Ciprofloxacin (5 µg); Nor = norfloxacin (10 µg); Ch = chloramphenicol (30 µg); Tet = tetracycline (30 µg); Ery= erythromycin (10 µg). (-) = no zone of inhibitions. IZD = inhibition zone diameter.

Statistically, the impact of the extract on the effect of Cip and Norfloxacin (Nor) against *E. coli* was significant but surprisingly not the effect on tetracycline (Tet) and Nor against the same *E. coli* ($p \geq 0.188$). The difference in the comparative effect of the extract on tetracycline and ciprofloxacin was significant against the same *E. coli* with

Table 7. Effects of extract of *P. nitida* on the activities of the antibiotic discs against antibiotic-resistant bacteria.

Organism	Number of isolates	Antibiotics	Mean IZD (mm)		Remarks
			Antibiotic disc alone	Antibiotic disc + Extract (1/4th MIC)	
E. coli	4	Cip	12	13	No effect (in 3 isolates)
		Nor	21	12	Antagonism (in all isolates)
		Tet	-	9	Synergism (in 3 isolates)
		Ery	-	-	No effect(in all isolates)
		Ch	-	11	Synergism(in all isolates)
P. aeruginosa	5	Cip	15	-	Antagonism (in all isolates)
		Nor	14	-	Antagonism(in all isolates)
		Tet	-	-	No effect (in all isolates)
		Ery	-	-	No effect (in all isolates)
		Ch	-	-	No effect (in 3 isolates)
S. aureus	4	Cip	22	16	Antagonism (in all isolates)
		Nor	15	9	Antagonism (in all isolates)
		Tet	-	-	No effect (in all isolates)
		Ery	-	-	No effect (in all isolates)
		Ch	12	14	Synergism (in 3 isolates)

Cip = Ciprofloxacin (5 µg); Nor = norfloxacin (10 µg); Ch = chloramphenicol (30 µg); Tet = tetracycline (30 µg); Ery= erythromycin (10µg). (-) = no zone of inhibitions. IZD = inhibition zone diameter.

the extract showing more synergistic effect on tetracycline. Between ciprofloxacin and norfloxacin against *P aeruginosa*, the difference was not significant (P ≥ 0.188).

The effects of the extract of *P. nitida* on the activities of the antibiotics against the antibiotic resistant bacteria in disc diffusion experiment and in macrobroth dilution tests are shown in Tables 7 and 8 respectively. The extract mostly exhibited antagonism with ciprofloxacin and norfloxacin in almost all the test organisms as evidenced by decrease in inhibition zone diameter of the antibiotic discs and increase in the MICs of the antibiotics. The extract however was synergistic with few antibiotics like chloramphenicol against resistant *E. coli* and *S. aureus.* ANOVA showed that the differences in effect of this extract on ciprofloxacin and tetracycline against *E. coli* was significant, antagonizing the former while enhancing the later. Between norfloxacin and tetracycline against *E. coli,* the difference was also significant but not between norfloxacin and ciprofloxacin (P ≥ 1.00) where it showed antagonism to the two against *S. aureus.*

DISCUSSION

The extracts of *C. odorata* and *A. africana* were able to potentiate the activities of some of the antibiotics against the antibiotic resistant bacteria isolates. Some researchers have suggested that the mechanism of the joint action of plant extracts and antibiotics could be as a result of perturbation of cell membrane and cell wall by the plant extract thereby increasing the influx of antibiotics into the bacterial cells (Sibanda and Okoh, 2007). However, some of the results obtained in this study suggested that some cases of synergism between the extracts and the antibiotics could be as a result of some of the phytochemicals that specifically react with and arrest some specific biochemical factors that mediate multiple drug resistance in the resistant bacteria. For example, the extract of *C. odorata* did not demonstrate any effect on ciprofloxacin and norfloxacin against antibiotic resistant isolates of E*. coli* and *S. aureus*, but the extract caused four-fold reduction in the MIC of ciprofloxacin and 8-fold reduction in MIC of norfloxacin against drug resistant *P. aeruginosa.* This suggests that the extract of *C. odorata* was able to inhibit a particular drug resistance mechanism in *P. aeruginosa,* but could not arrest the mechanisms of drug resistance in isolates of *E. coli* and *S. aureus*. *C. odorata* contains alkaloids, flavonoids, glycosides, saponins, tannins and terpenoids in equal properties according to our earlier finding (Eze et al., 2013). These substances, some of which are known to have antibacterial properties, appear to have had potentiating as well as antagonistic effects on some of these antibiotics depending on their chemical constituents.

Similar observation was also made where the extract of *A. africana* potentiated the activity of chloramphenicol by

Table 8. Effects of the extract of *P. nitida* on the MIC values of the antibiotics against the antibiotic resistant bacteria in macrobroth dilution test.

Organisms	Number of isolates	Antibiotic	MIC (µg/ml)		Remarks
			Antibiotic alone	Antibiotic + Extract (1/4th MIC)	
E. coli	3	Cip	5.3	32	Antagonism (in all isolates)
		Nor	8	64	Antagonism (in all isolates)
		Tet	32	8	Synergism (in all isolates)
		Ery	ND	ND	-
		Ch	> 128	53.3	Synergism (in all isolates)
P. aeruginosa	4	Cip	26.7	26.7	No effect (in all isolates)
		Nor	3.3	16	Antagonism (in all isolates)
		Tet	96	> 256	Antagonism (in 2 isolates)
		Ery	ND	ND	-
		Ch	> 128	56	Synergism (in all isolates)
S. aureus	3	Cip	< 0.125	0.5	Antagonism (in all isolates)
		Nor	3.3	24	Antagonism (in all isolates)
		Tet	24	3	Synergism (in 2 isolates)
		Ery	170.7	170.7	No effect (in all isolates)
		Ch	>128	10.6	Synergism (in 3 isolates)

Cip = Ciprofloxacin (5 µg); Nor = norfloxacin (10 µg); Ch = chloramphenicol (30 µg); Tet = tetracycline (30 µg); Ery= erythromycin (10 µg). (-) = no zone of inhibitions. IZD = inhibition zone diameter.

up to 4-fold reduction in MIC of the antibiotic against some resistant isolates of *P. aeruginosa*, but did not have any effect on the antibiotic against other organisms.

Inhibition of efflux pump proteins and inactivation of other enzymes mediating multidrug resistance in bacteria have been found to be the mechanisms by which some plant derived compounds augment the activity of antibiotics (Tegos et al., 2002*).*

It was also observed that effects of the plant extracts on the activities of the antibiotics varied in several cases among resistant bacteria isolates of the same species. For example, the extract of *A. africana* enhanced the activity of norfloxacin against 2 resistant isolates of *P. aeruginosa* out of the 4 resistant isolates tested (Tables 5 and 6). This suggests that, although resistant isolates belonged to the same species, they could be possessing different mechanisms of resistance and hence they responded differently to the combinatorial effects of plant extracts and antibiotics.

This study shows few cases of disharmony between the results of antibiotic extract combination in disc diffusion tests and in macro broth dilution tests. For example, the activity of tetracycline disc was enhanced by the extract of *C. odorata* against resistant *E. coli* isolates but the extract did not exhibit any effect on the MIC of tetracycline against these isolates in macrobroth dilution test (Tables 3 and 4). The reasons for these discrepancies are not very clear. However some researchers have postulated that synergistic effect of plant extract with antibiotics could be as a result of certain complex formations which become more effective in inhibition of a particular species of microorganisms either by inhibiting the cell wall synthesis or causing its lyses (Ahmed et al., 2010*)*. It may be rational to assume in the light of the above postulate, that the discrepancies observed between the result of disc diffusion test and macrobroth dilution, could be as a result of differences in the nature of complexes formed between the plant extracts and the antibiotics in the two test media (that is, the agar medium and the broth).

The *in vitro* combination of extract of *P. nitida* with ciprofloxacin and norfloxacin resulted in antagonism in almost all the test organisms. It seems that some constituents of the extracts are interfering with the active principles of the two antibiotics, probably by the formation of complexes that are inactive. In our earlier work (Eze et al., 2013) we reported that *P. nitida* contains alkaloids, flavonoids, tannins, terpenoids, saponins and steroids among other substances. These phytochemicals are capable of eliciting antibacterial activities and may be interfering with the bioactivities of the test antibiotics.

The extract of *P. nitida,* however exhibited synergistic interaction with tetracycline and chloramphenicol against resistant isolates of *E. coli*, but not against *P. aeruginosa* and *S. aureus*. Some researchers reported that the synergies detected in antibiotic-extract combination were

not specific to any group of organisms or class of antibiotics (Aiyegoro and Okoh, 2009). In contrast, the results of this study suggest some degree of specificities in synergism with respect to the type of organism and class of antibiotics.

Conclusion

The results of this study show that the extracts of such medicinal plants as *A. africana* and *C. odorata* can potentiate the activity of some antibiotics against antibiotic resistant bacteria. This synergism is statistically more pronounced with tetracycline and chloramphenicol when compared to others. Further studies that could isolate the active principles responsible for such resistance modulatory potency is recommended for development of standard resistance modifying agents.

However, the antagonistic reactions of the extract of *P. nitida* with norfloxacin and ciprofloxacin shows that indiscriminate co-administration of herbal drugs with standard antibiotics could be therapeutically wasteful. Their use in combination with conventional antibiotics should be further studied for *in vitro* activities. This may lead to the development of drug potentiating preparations that can be appropriately formulated and administered.

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