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Antibacterial resistance modulatory properties of selected medicinal plants from Ghana

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Bacterial resistance to antibiotics is a serious challenge to human and animal health and all efforts are being put together to resolve the menace. In this study the antibiotic resistance modifying activity of ten plants was established by determination of the minimum inhibitory concentrations (MICs) of the plant extracts, the MICs of the antibiotics alone (amoxicillin, ciprofloxacin, erythromycin and tetracycline) and the MICs of the antibiotics in the presence of sub-inhibitory concentrations of the methanol extracts of some selected medicinal plants including *Clerodendron splendens, Cyperus esculentus, Duranta plumieri, Kigelia africana, Kyllinga brevifolia, Momordica charantia, Phyllanthus amarus, Pycnanthus angolensis, Secamone afzelii* and *Thuja occidentalis* against two Gram-positive bacteria (*Staphylococcus aureus* and *Bacillus subtilis*) and two Gram-negative bacteria (*Escherichia coli* and *Pseudomonas aeruginosa*). The extracts were found to possess varying degree of antimicrobial activity with MICs between 4 and 50 mg/mL. It was observed that 26.9% of the plant extract-antibiotic combinations/interactions resulted in the reduction of activity of antibiotics. Almost 17% of the extract-antibiotic interactions led to the complete loss of activity of the antibiotics and 30.0% of the extract-antibiotic combinations resulted in resistance modulation. Three per cent of the extract-antibiotic combinations/interactions had antibiotics that were not active when used alone but became active in the presence of the extracts and 23.1% extract-antibiotic combinations/interactions had no modifying effect on the individual *in vitro* activities of the antibiotics. There is need to isolate the bioactive agents from the extracts especially those that potentiated the activity of the antibiotics.

Key words: Bacterial infections, antibiotic resistance, resistance modifying agents; medicinal plants.

INTRODUCTION

Antimicrobial resistance by pathogenic microorganisms (bacteria, viruses, fungi, protozoa and helminths) is one of the biggest clinical problems currently facing humanity (Davies and Davies, 2010). Drug combination, as exemplified by the combination of *β*-lactam antibiotics and *β*-lactamase inhibitors (for example amoxicillin–clavulanic acid) (Reeves et al*.,* 1978) is one way used to overcome resistance by pathogenic

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microorganisms. The mechanisms of action of such combinations vary overtly from that of the same drugs acting individually and isolation of a single phyto-constituent thus may alter its importance in exhibiting this effect (Hemaiswarya et al., 2008).

Nature has provided various sources of substances used as medicines and scientists have found medicines for almost all diseases known to man from natural sources (Newman et al., 2000). Mankind has been using antimicrobial agents for the management of infections for a longer period than initially anticipated (Bassett et al., 1980; Cook et al., 1989; Aminov, 2010).

Phylogenetic reconstruction analysis indicates that antibiotic resistance genes have been around long before the antibiotic era (Aminov and Mackie, 2007; Kobayashi et al., 2007). Structure-based phylogeny of serine and metallo-β-lactamases, for example, show that these ancient enzymes have been around for more than two billion years and that some serine β-lactamases have been present on plasmids for thousands of years (Hall and Barlow, 2004; Garau et al., 2005). The development of the β-lactamase and housekeeping genes in *Klebsiella oxytoca* is highly congruent, meaning that these genes have been evolving for over 100 million years (Fevre, 2005). Thus antibiotic resistance is not a new phenomenon, except that, probably it is now more widespread than before, and it moves round the globe more quickly because of easier and faster movement of mankind.

Antibiotic resistance has become a huge problem in recent times. Patients are dying from infections because the antibiotics used for the treatment of these infections are no more effective because of resistance. Resistance to antibiotics by bacteria and other organisms is now a major public health problem worldwide and antibiotic use has been recognized as the main selective pressure driving the menace.

Plants have been identified as one major source of medicinal agents from nature. Medicinal plants have been used by humans to manage several diseases including infections since time immemorial (Rios et al., 1988). Based on their traditional uses several known medicinal plants have been screened for their antimicrobial activity, phytochemical composition, possible interaction with antibiotics or other medicinal plants, pharmacokinetic, bioavailability and toxicity profiles. However, reports on interactions of antibiotics with plant extracts are scanty and only a few studies have been reported (Rios et al., 1988; Nascimento et al., 2000; Aburjai et al., 2001; Aqil et al., 2005). Natural product scientists have been searching for phytochemicals that could ultimately be developed for use in the treatment of infectious diseases (Cowan, 1999). The antiseptic use of volatile oils has been known to man for centuries and these oils have been obtained from plants using extraction methods such as steam or hydro-distillation. These volatile oils are usually variable mixtures of mostly terpenoids, specifically monoterpenes and sesquiterpenes although

diterpenes may also be present (Dorman and Deans, 2000).

Fractionation of *Glycyrrhiza glabra* L. var. typical, using bioactivity guided methods led to the isolation and characterization of several compounds including 4'-O-methylglabridin, 3'-methoxyglabridin, glabridin and glabrol. Glabrene, hispaglabridin A, hispaglabridin B, 3-hydroxyglabrol formononetin, phaseollinisoflavan, salicylic acid and O-acetyl salicylic acid were also found. Some of these including hispaglabridin A, hispaglabridin B, 4'-O-methylglabridin, glabridin, glabrol and 3-hydroxyglabrol were found to possess significant antimicrobial activity (Mitscher et al., 1980).

Apart from being sources of antimicrobial agents, plants also harbour substances that act to modify the resistance offered by microorganisms to antibiotics (Dapaah et al., 2016; Fankam et al., 2015; Seukep et al., 2016). In a study using the multidrug-resistant *Escherichia coli* that produces extended-spectrum β-lactamases (ESBLs), such as the CTX-M enzymes, the methanol extracts of *Gundelia tournefortii* L. and *Pimpinella anisum* L. enhanced the antibacterial activity of chloramphenicol, neomycin, doxycycline, cephalexin and nalidixic acid (Darwish and Aburjai, 2010). In this study, the antibacterial activity of selected plant extracts as well as their *in vitro* resistance modulatory effects on selected reference antibiotics were determined.

MATERIALS AND METHODS

Collection and identification of plant materials

The plants materials were identified, collected and authenticated by Dr. G. H. Sam of the Department of Herbal Medicine, Faculty of Pharmacy and Pharmaceutical Sciences, Kwame Nkrumah University of Science and Technology (KNUST), Kumasi, Ghana with voucher specimens kept at the herbarium of the above mentioned institution (Table 1).

Preparation of plant extracts

The various plant parts were washed under running tap water and air dried individually at room temperature (28 to 32°C) for 7 to 14 days (Meyer and Dilika, 1996; Bariş et al., 2006) to a constant weight. The dried plant materials were milled using a laboratory hammer mill into coarse powder. Each powdered plant material (100 g) was cold macerated with 500 mL of 70% v/v methanol for five (5) days with continuous stirring. They were then filtered using Whatman paper No.1 under reduced pressure. They were then evaporated to dryness using rotary evaporator under reduced pressure at 38°C, lyophilized and stored in a fridge at 4°C.

Antibacterial study

Determination of antibacterial activity of extracts

The antibacterial activities of the crude methanol extracts of the various plant parts were determined using the method as described by Das et al. (2011), Bhalodia and Shukla (2011) and Klančnik et al. (2010). One millilitre (1 mL) each of the test organism suspension (*S. aureus* ATCC 25923, *B. subtilis*

Table 1. List of collected medicinal plants.

Plants	Plant part used	Family	Voucher specimen number	Location	Geographical location	
					Latitude °	Longitude °
Clerodendron splendens G. Don.	Leaves	Lamiaceae	KNUST/HM/16/L/079	Asokore Mampong Kumasi	6.6990	-1.56914
Cyperus esculentus L.	Aerial parts	Cyperaceae	KNUST/HM/16/S/081	Achiase Nwabiagya, Kumasi	6.78535	-1.67474
Duranta plumieri L.	Leaves	Verbanaceae	KNUST/HM/16/L/082	KNUST, Kumasi	6.67650	-1.56698
Kigelia africana (Lam.) Benth.	Leaves	Bignoniaceae	KNUST/HM/16/L/083	Bobiri Forest, Kubease, Ashanti	6.68290	-1.36449
Kyllinga brevifolia Rottb.	Aerial parts	Cyperaceae	KNUST/HM/16/S/084	Boadi, Knust, Kumasi	6.67972	-1.54737
Momordica charantia L.	Leaves	Cucurbitaceae	KNUST/HM/16/L/085	Asokore Mampong, Kumasi	6.70032	-1.56874
Phyllantus amarus, Schumac & Thonn.	Aerial parts	Euphorbiaceae	KNUST/HM/16/S/088	KNUST, Kumasi	6.67649	-1.56700
Pycnanthus angolensis (Welw.) Warb.	Bark	Myristicaceae	KNUST/HM/16/B/089	KNUST, Kumasi	7.67375	-1.57763
Secamone afzelii, (Schult) K Schum.	Aerial parts	Asclepiadeceae	KNUST/HM/16/S/090	Physique Garden, KNUST, Kumasi	6.67384	-1.56627
Thuja occidentalis L.	Leaves	Cupressaceae	KNUST/HM/16/S/091	FPPS, KNUST, Kumasi	6.67649	-1.56699

NTCC 4853*, E.coli* ATCC 25922*, P. aeruginosa* ATCC 27853) was inoculated into a 10 mL nutrient broth (Oxoid, London UK) and incubated for 24 h and adjusted with normal saline to 0.5 McFarland Standard. Aliquots of 10 µL were applied to the surface of 20 mL of Mueller-Hinton agar (Sigma-Aldrich, Steinheim, Germany) in 10 cm-wide plates. The aliquot was spread over the surface using a sterile cotton wool swab. Three (3) cups were bored in each plate using a cork borer No. 5 (10 mm). Two grams of the extract was added to 1 mL of dimethyl sulfoxide (Sigma-Aldrich, Steinheim, Germany) and diluted to 200 mg/mL with sterile distilled water. It was then serially diluted and the wells filled with 100 µL of 30 and 50 mg/mL of the extract and allowed to diffuse at room temperature (25 to 28°C) for 1 h. A 10 µg/mL solution of tetracycline prepared in water was used as the positive control. The plates were incubated at 37°C for 24 h and zones of growth inhibition recorded. The procedure was performed in triplicates.

Determination of the minimum inhibitory concentration of extracts

The minimum inhibitory concentration (MIC) of all the plant extracts was determined using the macrobroth dilution method

(Okeke et al.*,* 2001; Wiegand et al.*,* 2008). Solutions of the extracts were prepared by mixing 4 g of extract with 0.5 mL of dimethylsulfoxide (DMSO) and 9.5 mL of distilled water added to produce a 400 mg/mL stock solution. Specified volumes (5 to 250 µL) were then taken from the stock solution and added to 0.5 mL double strength Muller-Hinton broth (Sigma-Aldrich, Steinheim, Germany). Appropriate volume of sterile distilled water was added and 10 µL of test organism containing 1 x 10^5 cells per mL was added to the appropriate well to produce concentrations of 2, 5, 10, 20, 30, 40, 50, 60, 70, 80, 90 and 100 mg/mL. They were incubated at 37°C for 24 h, after which 10 µL of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT 0.1% w/v) was added to each well to detect the presence of growth after 30 min of incubation at 37°C. The MICs were re-determined with a constant difference of 2 mg/mL.

The MICs of reference antibiotics (amoxicillin, erythromycin, tetracycline and ciprofloxacin) were similarly determined against *S. aureus, B. subtilis, E. coli* and *P. aeruginosa* using the broth microdilution method (Wiegand et al., 2008; Qaiyumi and Wanger, 2007). The reference antibiotics at 1, 2, 4, 8, 16, 32, 64 and 128 µg/mL were prepared in 96-well plates and the volumes adjusted to 190 µL and 10 µL of test organism suspension containing 10^5 cells per mL was added to make it

200 µL per well. They were incubated at 37°C for 24 h. The MIC was recorded as the least concentration that showed no visible bacterial growth which was detected by the absence of purple colour after the addition 10 µL of MTT (0.1% w/v) to each well followed by incubation at 37°C for 30 min.

Screening of extracts for antibacterial resistance modifying activities

The antibiotic resistance modifying activity was determined for all the extracts against the test organisms in the presence of the four reference antibiotics (amoxicillin, erythromycin, ciprofloxacin and tetracycline). The MIC of the reference antibiotics was determined against *S. aureus, B. subtilis, E. coli* and *P. aeruginosa* (as described above) in the presence of a sub-inhibitory concentration of 2 mg/mL of extracts by incorporating 10 µL of a 200 mg/mL stock solution of the extract into 100 µL of double strength Muller-Hinton broth. The broth was adjusted to 190 µL with sterile distilled water and 10 µL of test organism was added to produce 200 µL. The plates were then incubated at 37°C for 24 h. The MIC was recorded as the least concentration that showed no visible bacterial growth. This was detected by the absence of purple colour after the

Table 2. Antibacterial activity of plant extracts.

Plant	Part used	Mean zones of growth inhibition (mm)							
		S. aureus		*B. subtilis*		*E. coli*		*P. aeruginosa*	
Concentration of extract (mg/mL)		30.0	50.0	30.0	50.0	30.0	50.0	30.0	50.0
C. splendens	Leaves	17.3 ±0.33	19.6±0.33	15.6±0.33	18.6±0.33	14.0±1.00	16.6±0.66	11.3±0.33	14.3±0.33
C. esculentus	Aerial parts	11.6±0.33	14.3±0.33	13.7±0.67	15.7±0.88	14.3±0.33	16.7±0.33	-	12.6±0.33
D. plumieri	Leaves	-	-	12.0±0.58	14.7±0.88	-	12.0±0.58	-	-
K. Africana	Leaves	13.3±0.33	16.6±0.33	13.7±0.33	16.3±0.33	14.7±0.33	16.7±0.33	-	15.0±0.58
K. brevifolia	Aerial parts	-	12.3±0.67	-	13.0±1.15	12.7±0.33	15.7±0.33	-	13.3±0.88
M. charantia	Leaves	-	13.3±0.67	-	13.0±0.58	-	13.3±0.33	-	11.7±0.33
P. amarus	Aerial parts	16.3±0.33	19.3±0.33	14.3±0.33	17.7±0.88	12.3±0.33	15.0±1.00	12.6±0.33	15.6±0.88
P. angolensis	Bark	14.6±0.67	18.0±0.58	14.7±0.33	18.3±0.88	-	-	-	-
S. afzelii	Aerial parts	12.6±0.33	15.3±0.33	15.7±0.33	18.7±0.67	14.7±0.33	17.3±0.67	-	14.3±0.88
T. occidentalis	Leaves	19.6±0.33	23.3±0.33	13.7±0.33	16.3±0.67	14.7±0.67	18.0±0.58	14.0±0.58	16.7±67
Tetracycline (10 mg/mL)		25.8±0.55		32.33±1.4		25.8±0.55		32.33±1.4	

- = no activity

addition 10 µL of MTT (0.1% w/v) to each well followed by incubation at 37°C for 30 min. The experiment was done in three replicates.

RESULTS

Antibacterial activity of extracts

Antibacterial activity showed that the methanol leaf extract of *C. splendens*, methanol aerial parts extract of *P. amarus,* methanol leaf extract of *T. occidentalis* and aerial part methanol extract of *C. esculentus* had activity against all test organisms at test concentrations with zones of growth inhibition in the range of 11.3±0.33 to 19.6±0.33, 12.3±0.33 to 19.3±0.33, 13.7±0.33 to 19.6±0.33 and 11.6±0.33 to 15.7±0.88 mm, respectively (Table 2). Additionally, the methanol leaf extract of *K. africana*, methanol aerial part extract of *K. brevifolia*, methanol leaf extract of *M. charantia* and aerial part extract of *S. afzelii* exhibited activity against all the test organisms at 50 mg/mL. The methanol leaf extract of *D. plumieri* showed no activity against *S. aureus* and *P. aeruginosa* at test concentrations. Methanol bark extract of *P. angolensis* also showed no activity against test Gram-negative organisms (Table 2).

MIC of extracts

The Gram-positive bacteria were more susceptible to the extracts with lower MICs compared to the Gram-negative bacteria (Table 3). *P. amarus* and *C. splendens* were also very active against *B. subtilis* with MICs of 4 mg/mL. Methanol extracts of *M. charantia* and *K. brevifolia,* exhibited weak activity against *B. subtilis* with MICs of 36 and 20 mg/mL, respectively (Table 3). Methanol leaf extract of *T. occidentalis* had MIC of 4 mg/mL against *S. aureus*. *S. afzelii, M. charantia* and *K. brevifolia* extracts exhibited weak activity against *S. aureus* with MICs of 26, 30 and 32 mg/mL, respectively. *C. esculentus* showed moderate activity against *S. aureus* and *B. subtilis* with MIC of 16 mg/mL (Table 3).

E. coli was susceptible to the leaf extract of *P. amarus* and *C. splendens* with MICs of 4 mg/mL. The MIC of *C. esculentus* against *E. coli* was 8 mg/mL. Additionally, susceptibility of *E. coli* to extracts of *M arantia* and *D. plumieri* was quite low with MICs of 32

Table 3. MIC of methanol extracts against test organisms.

Plant	Part used	MIC (mg/mL)			
		S. aureus	*B. subtilis*	*E. coli*	*P. aeruginosa*
C. splendens	Leaves	8.0	4.0	4.0	26
C. esculentus	Aerial parts	16.0	16.0	8.0	32.0
D. plumieri	Leaves	>100.0	12.0	48.0	>100
K. africana	Leaves	16.0	8.0	10.0	22.0
K. brevifolia	Aerial parts	32.0	20.0	14.0	8.0
M. charantia	Leaves	30.0	36.0	32.0	38.0
P. amarus	Aerial parts	6.0	4.0	4.0	6.0
P. angolensis	Bark	8.0	6.0	>100	>100
S. afzelii	Leaves	26.0	8.0	6.0	22.0
T. occidentalis	Leaves	4.0	8.0	6.0	10.0

and 48 mg/mL, respectively. *P. aeruginosa* was comparably, the most resistant organism to all the extracts but was most sensitive to *P. amarus* with MIC of 6 mg/mL. It showed very little susceptibility to *C. esculentus*, *S. afzelii*, *M. charantia*, *K. africana* and *C. splendens* with MICs of 32, 22, 38, 22, 32 and 26 mg/mL, respectively (Table 3).

Some of the extracts did not show activity at all against the test organisms within the range of concentrations used. The methanol extract of *D. plumieri* was not active against *S. aureus* and *P. aeruginosa* while *P. angolensis* showed no activity against the Gram-negative bacteria (Table 3).

Antibiotic resistance modifying/modulation activity of extracts

Influence of C. splendens extract on activity of selected antibiotics

Sub-inhibitory concentrations of methanol leaf extract of *C. splendens* had minimal effects on the activities of the antibiotics (Table 4). The extract enhanced the activity of tetracycline against *P. aeruginosa* by reducing the MIC by 8 folds (Table 14). Most of the interactions between the organisms and the antibiotics in the presence of the sub-inhibitory concentration of methanol extracts of *C. splendens* resulted in marginal changes in their MICs (Tables 4 and 14).

Influence of C. esculentus extract on activity of some selected antibiotics

The MICs of the reference antibiotics were determined with and without the sub-inhibitory concentration (2.0 mg/mL) of methanol aerial parts extract of *C. esculentus.* It was established that the antibacterial activities of all the reference antibiotics against all the

organisms were reduced (Tables 5 and 14).

Influence of D. plumieri extract on activity of selected antibiotics

The presence of sub-inhibitory concentration of methanol leaf extract *D. plumieri* (2 mg/mL) reduced the activity of the antibiotics against most of the test organisms (Table 6). With the exception of *E. coli, S. aureus* and *P. aeruginosa* that saw increased sensitivity to ciprofloxacin, erythromycin and tetracycline producing 2.5, 2 and 8-folds reduction in resistance respectively almost all the interactions produced resistance enhancement (Table 14).

Influence of K. africana extract on activity of selected antibiotics

Methanol leaf extracts of *K. africana* modulated the susceptibility of the test organisms to the antibiotics. The resistance of *B. subtilis* reduced against all the antibiotics with amoxicillin showing the greatest increase in activity producing a 15-fold decrease in MIC. In the presence of the extract, *E. coli* lost sensitivity to all the antibiotics. The sensitivity of *P. aeruginosa* to tetracycline increased by 8-fold (Tables 7 and 14).

Influence of K. brevifolia extract on activity of selected antibiotics

The methanol aerial parts of extract of *K. brevifolia* nullified the activity of amoxicillin against *S. aureus* and *B. subtilis* and also tetracycline against *B. subtilis*. The extract made *P. aeruginosa* sensitive to amoxicillin with an MIC of 32 μg/mL, while producing 2 and 4 fold enhancement in the activities of erythromycin and

Table 4. Influence of *C. splendens* extract on activity of selected antibiotics.

Organism	Extract (mg/mL)	Amoxicillin (µg/mL)		Ciprofloxacin (µg/mL)		Erythromycin (µg/mL)		Tetracycline (µg/mL)	
		A	B	A	B	A	B	A	B
SA	8.0	2.0	2.0	1.0	1.0	2.0	2.0	2.0	2.0
BS	4.0	2.0	4.0	0.5	1.0	1.0	2.0	2.0	4.0
EC	4.0	4.0	2.0	0.5	0.5	4.0	4.0	4.0	4.0
PA	26.0	>128.0	>128.0	2.0	1.0	>128.0	>128.0	32.0	4.0

Sub-inhibitory concentration of extract: 2.0 mg/mL, **A**: MIC of antibiotic alone (µg/mL), **B**: MIC of antibiotic in presence of sub-inhibitory concentration of extract (µg/mL). SA, *S. aureus*, BS, *B. subtilis*; EC, *E. coli*; PA, *P. aeruginosa*

Table 5. Influence of *C. esculentus* extract on activity of selected antibiotics.

Organism	Extract (mg/mL)	Amoxicillin (µg/mL)		Ciprofloxacin (µg/mL)		Erythromycin (µg/mL)		Tetracycline (µg/mL)	
		A	B	A	B	A	B	A	B
SA	16.0	2.0	4.0	1.0	4.0	2.0	16.0	2.0	32.0
BS	16.0	2.0	>128.0	0.5	>128	1.0	>128.0	2.0	>128.0
EC	8.0	4.0	32.0	0.5	2.0	4.0	8.0	4.0	32.0
PA	32.0	>128.0	>128.0	2.0	>128	>128.0	>128.0	32.0	>128.0

Sub-inhibitory concentration of extract: 2.0 mg/mL, **A**: MIC of antibiotic alone (µg/mL), **B**: MIC of antibiotic in presence of sub-inhibitory concentration of extract (µg/mL). SA, *S. aureus;* BS, *B. subtilis*; EC, *E. coli*; PA, *P. aeruginosa.*

Table 6. Influence of *D. Plumieri* extract on activity of selected antibiotics.

Organism	Extract (mg/mL)	Amoxicillin (µg/mL)		Ciprofloxacin (µg/mL)		Erythromycin (µg/mL)		Tetracycline (µg/mL)	
		A	B	A	B	A	B	A	B
SA	>100.0	2.0	2.0	1.0	16.0	2.0	1.0	2.0	8.0
BS	12.0	2.0	4.0	0.5	16.0	1.0	>128.0	2.0	4.0
EC	48.0	4.0	4.0	0.5	2.0	4.0	8.0	4.0	4.0
PA	>100.0	>128.0	>128.0	2.0	8.0	>128.0	>128.0	32.0	4.0

Sub-inhibitory concentration of extract: 2.0 mg/mL, **A**: MIC of antibiotic alone (µg/mL), **B**: MIC of antibiotic in presence of sub-inhibitory concentration of extract (µg/mL). SA, *S. aureus* BS, *B. subtilis*; EC, *E. coli*; PA, *P. aeruginosa*

tetracycline against E. coli, respectively Tables 8 and 14).

Influence of *M. charantia* extract on activity of selected antibiotics

In the presence of sub-inhibitory concentration (2 mg/mL) of methanol leaf of M. charantia, S. aureus and B. subtilis lost their sensitivity to tetracycline completely (Table 9). The resistance of S. aureus was reduced 66 folds to amoxicillin while that of P. aeruginosa and E. coli were reduced 15 and 13 folds with MICs of 0.13 and 0.3 µg/mL, respectively (Table 14).

Influence of *P. amarus* extract on activity of selected antibiotics

In the presence of sub-inhibitory concentration (2 mg/mL) of *P. amarus,* the organisms were more resistant to amoxicillin with higher MICs than theamoxicillin alone. With the exception of *S. aureus,* all the organisms were more sensitive to ciprofloxacin in the presence of the extract. Erythromycin lost activity completely against *S. aureus* rather had lower MIC of 0.13 µg/mL against *P. aeruginosa* (Table 10). With the exception of *S. aureus*, the rest of the organisms including *B. subtilis, E. coli* and *P. aeruginosa* became more sensitive to tetracycline with 4, 8 and 16 folds

Table 7. Influence of *K. africana* extract on activity of selected antibiotics.

Organism	Extract (mg/mL)	Amoxicillin (µg/mL)		Ciprofloxacin (µg/mL)		Erythromycin (µg/mL)		Tetracycline (µg/mL)	
		A	B	A	B	A	B	A	B
SA	16.0	2.0	1.0	1.0	1.0	2.0	0.5	2.0	2.0
BS	8.0	2.0	0.13	0.5	0.13	1.0	0.5	2.0	0.3
EC	10.0	4.0	>128.0	0.5	>32.0	4.0	>128.0	4.0	>128.0
PA	22.0	>128.0	>128.0	2.0	1.0	>128.0	>128.0	32.0	4.0

Sub-inhibitory concentration of extract: 2.0 mg/mL, **A**: MIC of antibiotic alone (µg/mL), **B**: MIC of antibiotic in presence of sub-inhibitory concentration of extract (µg/mL). SA, *S. aureus;* BS, *B. subtilis*; EC, *E. coli*; PA, *P. aeruginosa.*

Table 8. Influence of *K. brevifolia* extract on the antibacterial activity of selected antibiotics.

Organism	Extract (mg/mL)	Amoxicillin (µg/mL)		Ciprofloxacin (µg/mL)		Erythromycin (µg/mL)		Tetracycline (µg/mL)	
		A	B	A	B	A	B	A	B
SA	32.0	2.0	>128.0	1.0	1.0	2.0	64.0	2.0	2.0
BS	20.0	2.0	>128.0	0.5	0.3	1.0	32.0	2.0	>128.0
EC	14.0	4.0	128.0	0.5	1.0	4.0	2.0	4.0	1.0
PA	8.0	>128.0	32.0	2.0	2.0	>128.0	>128.0	32.0	64.0

Sub-inhibitory concentration of extract: 2.0 mg/mL, **A**: MIC of antibiotic alone (µg/mL), **B**: MIC of antibiotic in presence of sub-inhibitory concentration of extract (µg/mL). SA, *S. aureus;* BS, *B. subtilis*; EC, *E. coli*; PA, *P. aeruginosa.*

Table 9. Influence of *M. charantia* extract on activity of selected antibiotics.

Organism	Extract (mg/mL)	Amoxicillin (µg/mL)		Ciprofloxacin (µg/mL)		Erythromycin (µg/mL)		Tetracycline (µg/mL)	
		A	B	A	B	A	B	A	B
SA	30.0	2.0	0.03	1.0	1.0	2.0	1.0	2.0	>128.0
BS	36.0	2.0	2.0	0.5	0.13	1.0	0.13	2.0	>128.0
EC	32.0	4.0	2.0	0.5	0.13	4.0	0.5	4.0	0.3
PA	38.0	>128.0	>128.0	2.0	0.13	>128.0	>128.0	32.0	128.0

Sub-inhibitory concentration of extract: 2.0 mg/mL, **A**: MIC of antibiotic alone (µg/mL), **B**: MIC of antibiotic in presence of sub-inhibitory concentration of extract (µg/mL). SA, *S. aureus* BS, *B. subtilis*; EC, *E. coli*; PA, *P. aeruginosa.*

reduction in MICs respectively (Table 14).

Influence of *P. angolensis* extract on activity of selected antibiotics

Methanol bark extract of *P. angolensis* had marginal effects on the activities of the antibiotics (Table 11). Amoxicillin, ciprofloxacin and erythromycin were more active against *S. aureus* with 2-fold reduction of their MICs. In the presence of the extract, amoxicillin and ciprofloxacin were less active against *B. subtilis* (Table 14).

Influence of *S. afzelii* extract on activity of selected antibiotics

P. aeruginosa was very sensitive to amoxicillin when combined with methanol leaf extract of *S. afzelii* with an MIC of 16 µg/mL while amoxicillin alone at a concentration of 128 µg/mL had no effect on *P. aeruginosa* (Table 12). *S. aureus*, *B. subtilis* and *P. aeruginosa* also became less resistant to ciprofloxacin in the presence of the extract. The extract made all the organisms more resistant to erythromycin except *P. aeruginosa* that became very sensitive to the antibiotic in the presence of the extract with MIC of 8 µg/mL while

Table 10. Influence of *P. amarus* extract on activity of selected antibiotics.

Organism	Extract (mg/mL)	Amoxicillin (µg/mL)		Ciprofloxacin (µg/mL)		Erythromycin (µg/mL)		Tetracycline (µg/mL)	
		A	B	A	B	A	B	A	B
SA	6.0	2.0	8.0	1.0	2.0	2.0	>128.0	2.0	>128.0
BS	4.0	2.0	4.0	0.5	0.13	1.0	0.13	2.0	0.5
EC	4.0	4.0	32.0	0.5	0.13	4.0	8.0	4.0	0.5
PA	6.0	>128.0	>128.0	2.0	0.3	>128.0	0.13	32.0	2.0

Sub-inhibitory concentration of extract: 2.0 mg/mL, **A**: MIC of antibiotic alone (µg/mL), **B**: MIC of antibiotic in presence of sub-inhibitory concentration of extract (µg/mL). SA, *S. aureus* ; BS, *B. subtilis*; EC, *E. coli*; PA, *P. aeruginosa.*

Table 11. Influence of *P. angolensis* extract on activity of selected antibiotics.

Organism	Extract (mg/mL)	Amoxicillin (µg/mL)		Ciprofloxacin (µg/mL)		Erythromycin (µg/mL)		Tetracycline (µg/mL)	
		A	B	A	B	A	B	A	B
SA	8.0	2.0	1.0	1.0	0.5	2.0	1.0	2.0	4.0
BS	6.0	2.0	4.0	0.5	1.0	1.0	0.5	2.0	2.0
EC	-	4.0	8.0	0.5	1.0	4.0	4.0	4.0	4.0
PA	-	>128.0	>128.0	2.0	0.5	>128.0	>128.0	32.0	32.0

Sub-inhibitory concentration of extract: 2.0 mg/mL, **A**: MIC of antibiotic alone (µg/mL), **B**: MIC of antibiotic in presence of sub-inhibitory concentration of extract (µg/mL). SA, *S. aureus;* BS, *B. subtilis*; EC, *E. coli*; PA, *P. aeruginosa;* - = no activity.

Table 12. Influence of *S. afzelii* extract on activity of selected antibiotics.

Organism	Extract (mg/mL)	Amoxicillin (µg/mL)		Ciprofloxacin (µg/mL)		Erythromycin (µg/mL)		Tetracycline (µg/mL)	
		A	B	A	B	A	B	A	B
SA	26.0	2.0	1.0	1.0	0.13	2.0	4.0	2.0	>128.0
BS	8.0	2.0	1.0	0.5	0.13	1.0	>128.0	2.0	1.0
EC	6.0	4.0	0.5	0.5	0.5	4.0	>128.0	4.0	>128.0
PA	22.0	>128.0	16.0	2.0	0.3	>128.0	8.0	32.0	8.0

Sub-inhibitory concentration of extract: 2.0 mg/mL, **A**: MIC of antibiotic alone (µg/mL), **B**: MIC of antibiotic in presence of sub-inhibitory concentration of extract (µg/mL). SA, *S. aureus;* BS, *B. subtilis*; EC, *E. coli*; PA, *P. aeruginosa.*

the antibiotic alone has no activity within the concentrations used. Methanol leaf extract of *S. afzelii* demonstrated resistance modulation activity on amoxicillin against all the test organisms with 2-fold potentiation of amoxicillin against *S. aureus* and *B. subtilis* and an 8-fold potentiation against *E. coli*. In the case of tetracycline, *S. aureus* and *E. coli* lost their sensitivity completely while *B. subtilis* and *P. eruginosa* became more susceptible in the presence of the extract (Table 14).

Influence of *T. occidentalis* extract on activity of selected antibiotics

The methanol leaf extract of *T. occidentalis* suppressed the activity of all the antibiotics against all the test organisms except in some few cases. *S. aureus* and *B. subtilis* lost sensitivity completely to amoxicillin while *S. aureus, E. coli* and *P. aeruginosa* lost their sensitivity completely to tetracycline in the presence of the extract. *B. subtilis* showed a 33 folds reduction in resistance to

Table 13. Influence of *T. occidentalis* extract on activity of selected antibiotics.

Organism	Extract (mg/mL)	Amoxicillin (µg/mL)		Ciprofloxacin (µg/mL)		Erythromycin (µg/mL)		Tetracycline (µg/mL)	
		A	B	A	B	A	B	A	B
SA	4.0	2.0	>128.0	1.0	32.0	2.0	16.0	2.0	>128.0
BS	8.0	2.0	>128.0	0.5	32.0	1.0	0.03	2.0	0.06
EC	6.0	4.0	8.0	0.5	32.0	4.0	0.5	4.0	>128.0
PA	10.0	>128.0	>128.0	2.0	32.0	>128.0	1.0	32.0	>128.0

Sub-inhibitory concentration of extract: 2.0 mg/mL, **A**: MIC of antibiotic alone (µg/mL), **B**: MIC of antibiotic in presence of sub-inhibitory concentration of extract (µg/mL). SA, *S. aureus;* BS, *B. subtilis*; EC, *E. coli*; PA, *P. aeruginosa*

Table 14. Ratio of MIC of antibiotic alone to MIC of antibiotic in the presence of sub-inhibitory concentration of extract.

Plant extract	Amoxicillin				Ciprofloxacin				Erythromycin				Tetracycline			
	SA	BS	EC	PA	SA	BS	EC	PA	SA	BS	EC	PA	SA	BS	EC	PA
C. splendens	-	0.5	2	-	-	0.5	-	2	-	0.5	-	-	-	0.5	-	8
C. esculentus	0.5	↓	0.125	-	0.25	↓	0.25	↓	0.13	↓	0.5	-	0.06	↓	0.13	↓
D. plumieri	-	0.5	-	-	0.06	0.03	2.5	0.25	2	↓	0.5	-	0.25	0.5	-	8
K. africana	2	15.38	↓	-	-	3.85	↓	2	4	2	↓	-	-	6.67	↓	8
K. brevifolia	↓	↓	0.03	↑	-	1.67	0.5	-	0.03	0.03	2	-	-	↓	4	0.5
M. charantia	66.7	-	2	-	-	3.85	3.85	15.39	2	7.69	0.8	-	↓	↓	13.33	0.25
P. amarus	0.25	0.5	0.13	-	0.5	3.85	3.85	6.67	↓	7.69	0.5	↑	↓	4	8	16
P. angolensis	2	0.5	0.5	-	2	0.5	0.5	4	2	2	-	-	0.5	-	-	-
S. afzelii	2	2	8	↑	7.69	3.85	-	6.67	0.5	↓	↓	↑	↓	2	↓	4
T. occidentalis	↓	↓	0.5	-	0.03	0.02	0.02	0.06	0.13	33.33	8	↑	↓	33.33	↓	↓

SA, *S. aureus*; BS, *B. subtilis*; EC, *E. coli*; PS, *P. aeruginosa*; ↓= antagonism; ↑= potentiation; - = no activity.

erythromycin and tetracycline while *E. coli* also showed an 8 folds reduction in resistance to erythromycin. *P. aeruginosa* became more sensitivity to erythromycin in the presence of the extract with an MIC of 1 µg/mL (Tables 13 and 14).

Ratio of activity of antibiotic to activity of antibiotic in the presence of extract

The antibacterial activity of the extracts and reference antibiotics were expressed as MIC, the smaller the MIC the more active the substance. Thus the effect of the sub-inhibitory concentration of the extracts on the activity of the test antibiotics was determined by comparing the ration of MIC of the antibiotic to the MIC of the antibiotic in the presence of sub-inhibitory concentrations of the extract (Table 14).

DISCUSSION

Some plant extracts have been shown over the years to exhibit antimicrobial activity and hence the agar diffusion method was employed to determine the activity of the selected plant extracts against test Gram-positive and negative bacteria. The study revealed that the methanol leaf extract of *C. splendens* (11.3±0.33 - 19.6±0.33 mm), methanol aerial part extract of *P. amarus* (12.3±0.33 - 19.3±0.33 mm)*,* methanol leaf extract of *T. occidentalis* (13.7±0.33 to 19.6±0.33 mm) and aerial part methanol extract of *C. esculentus* (11.6±0.33 to 15.7±0.88 mm) had activity against all test organisms at test concentrations (Table 2). Methanol leaf extract of *C. splendens* have been reported to have antibacterial and antiprotozoan activity, the former is in agreement with our studies (Gbedema et al.*,* 2010; Abouzid et al.*,* 2013). MIC of *C. splendens* methanol extract was determined to be 4.0 to 26 mg/mL against test Gram-positive and negative organisms (Table 3). However, Gbedema et al. (2010) reported a MIC of 128 to 516 µg/mL for extract of *C. splendens,* indicating a better antimicrobial activity than our findings. The difference may be due to method of extraction, the part of the plant used, site of collection and variation in storage conditions may contribute to the variations in activity (Inbathamizh and Padmini, 2013; Khattak, 2015). The extract of *C. splendens*

enhanced the activity of tetracycline against *P. aeruginosa* by reducing the MIC by 8 folds (Table 14). This reduction may due to blockade of tetracycline efflux pump activity or modification of the binding site which have been identified as the main mechanism of bacteria resistance to antibiotics including the tetraclyclines (John, 2005; Lin et al., 2015).

Aerial parts extract of *C. esculentus* showed antibacterial activity with zones of inhibition and MIC determined to be between 11.6±0.33 to 16.7±0.33 mmand 8 to 32 mg/mL, respectively for test organisms. This is agreement with the findings of Adeniyi et al. (2014) in which the zone of inhibition was reported to be within the range of 7.5±0.41 to 25.0±0.16 mm for aqueous and ethanol whole plant extracts of *C. esculentus*. It was also established that the antibacterial activities of all the antibiotics against all the organisms were reduced (Tables 5 and 14). This may probably be due to the extracts ability to reduce efflux pump activity, increase drug uptake and accumulation (Lin et al., 2015; Munita and Arias, 2016).

In the case of *D. plumieri* leaf extract, the zones of inhibition were determined to be 12.0±0.58 and 14.7±0.88 mm for *B. subtilis*. No activity was observed for *S. aureus* and *P. aeruginosa* but for *E. coli* (12.0±0.58 mm) at a concentration of 50 mg/mL. This indicates that the extract has weak antibacterial activity which is in agreement with the findings of Adu et al. (2011). The presence of sub-inhibitory concentration of *D. plumieri* leaf extract reduced the activity of the antibiotics against most of the test organisms (Tables 6 and 14). The reduced or nullified activity of the antibiotics may be as a result of interactions between the phytochemicals in the extract and the antibiotics or the microorganisms. The phyto-constituents may react chemically with the antibiotics leading to loss of activity (Dapaah et al., 2016; Adu et al., 2009).

The methanol leaf extract of *K. africana* showed activity against test organisms which is consistent with report by Agyare et al. (2013). The ethanolic extracts of *K. africana* stem bark and fruit extracts have also been reported to possess activity against *B. subtilis, E. coli, K. pneumoniae* and *S. aureus* with MICs of 0.63 to 2.5 mg/mL (Grace et al., 2002). The resistance of *B. subtilis* was reduced against all the antibiotics with amoxicillin showing the greatest increase in activity producing a 15-fold reduction in the MIC. Sensitivity of *P. aeruginosa* to tetracycline increased by 8-folds. This observed potentiation in activities of the antibiotics may be due to increased drug uptake and its accumulation in bacteria (Lin et al., 2015; Munita and Arias, 2016).

Extracts of *K. brevifolia* nullified the antibacterial activity of amoxicillin against *S. aureus* and *B. subtilis* and also tetracycline against *B. subtilis* which may be due to the phyto-constituents reacting chemically with the antibiotics leading to loss of activity (Dapaah et al., 2016; Adu et al., 2009). The extract enhanced the sensitivity of *P. aeruginosa* to amoxicillin with an MIC of 32 µg/mL, while producing 2 and 4 fold potentiation of

the activities of erythromycin and tetracycline respectively, against *E. coli* (Tables 8 and 14). This indicates that the extract possesses phyto-constituents that make the organisms more susceptible to the antibiotics which may be due to increased drug uptake and efflux pump activity inhibition as well as β lactamase inhibition in the case of penicillins (Munita and Arias, 2016; Adu et al., 2009).

For *M. charantia*, the MIC and zones of growth inhibition was respectively determined to be in the range of 30 to 38 mg/mL and 11.7±0.33 to 13.3±0.33 mm against test organisms. These findings are in agreement with the reported antimicrobial activity of leaf, seed and fruit extracts of *M. charantia* (Costa et al., 2010; Ozusaglam et al., 2013; de Lucena et al., 2015). In the presence of sub-inhibitory concentration of *M. charantia* extract, *S. aureus* and *B. subtilis* lost their sensitivity to tetracycline completely (Table 9). The resistance of *S. aureus* was reduced 66 folds to amoxicillin while that of *P. aeruginosa* and *E. coli* were reduced 15 and 13 folds with MICs of 0.13 and 0.3 µg/mL, respectively (Table 14). This indicates that the extract contains phytochemicals that have either synergistic or inhibitory effect when combined with the test antibiotics (Adu et al., 2009; Costa et al., 2010).

The aerial part methanol extract of *P. amarus* was determined to possess antibacterial activity with MIC in the range of 4 to 6 mg/mL which is in agreement with the findings of Babatunde et al. (2014) and Oluwafemi and Debiri (2008). With the exception of *S. aureus,* the test organisms were more sensitive to ciprofloxacin in the presence of the extract indicating a lowering of efflux pump activity and reduced drug uptake which are the major players in bacterial resistance to the flouroquinolones (Munita and Arias, 2016; Fabrega et al., 2009). With the exception of *S. aureus* that lost activity completely in the presence of the extract *B. subtilis, E. coli* and *P. aeruginosa* became more sensitive to tetracycline with 4, 8 and 16-folds reduction in MICs, respectively (Tables 10 and 14). The observed effect may due to blockade of tetracycline efflux pump activity or modification of the binding site which have been identified as the main mechanism of bacteria resistance to tetracycline (John, 2005; Munita and Arias, 2016).

The methanol bark extract of *P. angolensis* was active against only *S. aureus* and *B subtilis* but not *E. coli* and *P. aeruginosa*. The findings of Olademeji et al. (2006) which give credence to our studies indicate that the stem, leaves and root bark extracts of *P. angolensis* have activity against Gram-positive bacteria but not Gram-negative bacteria. However, Kuete et al. (2011) reported that the root bark extract has antimicrobial activity. In the presence of the extract *B. subtilis* became more resistant to amoxicillin and ciprofloxacin with the resistance increasing 2 folds (Tables 11 and 14). However, Lambert (2002) reported that some phyto-constituents may act as protein activators or co-enzymes which bind to and activate enzymes or genes

responsible for resistance in an organism.

Methanol aerial part extract of *S. afzelii* was observed have antibacterial activity which is in agreement with previous studies (Lagnika et al*., 2011). The observance of both resistance and synergistic effects from the methanol aerial part extract of *S. afzelii* (Tables 12 and 14) may be due the presence of myriad compounds. These compounds may either potentiate or inhibit the activity of the test antibiotics by activating or inhibiting certain enzymes or genes responsible for their resistance (Dapaah et al*., 2016; Adu et al*., 2009).

Methanol leaf extract of *T. occidentalis* had antibacterial activity with MIC of 4 to 10 mg/mL which is in agreement with the findings of Eltayeb and Hamid (2017). However, Sah et al. (2017) reported better antibacterial activity for *T. occidentalis* extract which may be due to the solvent system (a mixture of ethylacetate chloroform and ethanol in a ratio of 30:30:40) used in their extraction protocol. Additionally, Khubeiz et al*. (2016) reported a poorer antibacterial activity of *T. occidentalis* compared to our findings which may be due to method of extraction (aqueous extraction) and also the geographical as well as the season of collection of plant material which have been reported to influence the levels of the various secondary metabolites in the plants (Inbathamizh and Padmini, 2013; Khattak, 2015). Additionally, *B. subtilis* showed a 33-fold reduction in resistance to erythromycin and tetracycline while *E. coli* also showed an 8 folds reduction in resistance to erythromycin. *P. aeruginosa* gained sensitivity to erythromycin in the presence of the extract with an MIC of 1 µg/mL (Tables 13 and 14). The increased sensitivity of the test organisms to erythromycin and tetracycline may be due to decreased efflux pump activity, target site modification and reduce drug uptake (John, 2005; Munita and Arias, 2016; Leclercq and Courvalin, 2002).

In general, there was almost 30% reduction in *in vitro* activities of the antibiotics when they were combined with the plant extracts. This means that the sub-inhibitory concentrations of the extracts inhibited some mechanisms of action of the antibiotics. In some situations, the antibiotic alone had activity against the organism but in the presence of the extract, the activity of the antibiotic was lost completely thus making the organism very resistant to the antibiotic. This may mean that, the extract might have prevented the antibiotic from entering the organism, prevented the antibiotic from reaching its target site after entering the organism, reacted with the antibiotic to produce a non-active compound(s) or, inhibited one or more enzymes that were involved in the mechanisms of antimicrobial action (Dapaah et al*., 2016; Adu et al*., 2009; Adu et al*., 2014).

The study clearly shows that when medicinal plants are combined with antibiotics, microbial resistance which is a global problem can be reduced. Synergistic combinations of medicinal plants and antibiotics may help to reduce emergence of multidrug resistant mutants, toxicity, exhibit more antimicrobial activity and more effective against mixed infections (Bhardwaji et al*., 2016; Abascal and Yarnell, 2004). The above findings show the need to exercise caution when it comes to the indiscriminate combination of herbal medicines with antibiotics.

Conclusion

Potentiation and antagonistic activities were observed among the plant extract-antibiotic combinations. Synergistic activity observed from combinations of plant extract and antibiotics could help reduce the emergence and effect of multidrug resistant strains responsible for some bacterial infections and these extracts possess phytochemicals which could be exploited for their synergistic or antagonistic effect when combined with antibiotics. It is recommended that further studies be carried out to identify the phytocompounds responsible for the observed potentiation or antagonism when these plant extracts are combined with the selected reference antibiotics.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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