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ENHANCEMENT OF ANTIBACTERIAL ACTIVITY OF AMOXICILLIN BY SOME GHANAIAN MEDICINAL PLANT EXTRACTS

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ABSTRACT

As part of our ongoing study to screen local herbs for their possible usefulness as anti-infectives, we assessed extracts from 16 medicinal plants for their antibacterial properties and their influence on the activity of amoxicillin. The minimum inhibitory concentrations (MIC) of amoxicillin against *Staphylococcus aureus, Bacillus subtilis, Pseudomonas aeruginosa, Escherichia coli* and *Salmonella typhi* were determined alone and in the presence of sub-inhibitory concentrations of the extracts by the Kirby–Bauer agar diffusion method of antibacterial assay. Eleven out of 18 extracts exhibited antibacterial activity with MIC values below 20 mg/ml against at least one of the test bacteria employed. Amoxicillin activity against *Staph. aureus* was significantly ($p<0.05$) enhanced by the presence of sub-inhibitory concentrations of 5 extracts (*Mallotus oppositifolius, Bidens pilosa, Morinda lucida, Croton membranaceus* and *Jatropha curcas*). *B. subtilis* also became significantly susceptible to amoxicillin in the presence of 10 µg/ml extracts of *B. pilosa, Hibiscus sabdariffa*, *M. oppositifolius, Momordica charantia, Anoclesta nobilis, Cryptolepis sanguinolenta* and *Moringa oleifera*. *Spathodia campanulata, M. lucida, M. oleifera* and *J. curcas* leaf extracts also significantly reduced the MIC of amoxicillin against *E. coli* while *S. typhi* susceptibility was enhanced by the presence of *A. nobilis*, *M. charantia* and *J. curcas* extracts. We hereby report that sub-inhibitory concentrations of some plant extracts can enhance amoxicillin activity and these plants may provide lead compounds that may serve as cheap alternative adjuvants to clavulanic acid in amoxicillin formulations for the treatment of resistant opportunistic bacterial infections usually encountered among HIV/AIDS patients.

INTRODUCTION: Traditional medicine is the most accessible healthcare option in Ghana and other third world countries for the management of various disease conditions and this knowledge has served as clue to scientific investigation of many folklore herbs for bioactive phytochemicals. The discovery of bioactive compounds from plant sources is well documented; the phytoconstituents of garlic, tea, cinchona and lemon have been shown to exhibit broad spectrum antimicrobial activity [1]. Compounds from *Vitex rotundifolia* and *Arnebia euchroma* have also demonstrated excellent antibacterial activity against Methicillin-Resistant *Staphylococcus aureus* [2].

Phytoconstituents represent a large untapped reservoir of molecules with diverse chemical structures which are not simply based on existing antibiotic templates [3, 4, 5]. The increasing incidence of antibiotic resistant bacterial infections especially among HIV/AIDS patients has led to a surge in the investigation of phytoconstituents for their possible application in chemotherapy. These compounds may therefore play a vital role as leads for very potent anti-infective drugs needed in chemotherapeutic management of the antibiotic resistant bacterial infections [6].

Amoxicillin is a moderate-spectrum, bacteriolytic β-lactam antibiotic used to treat bacterial infections [7]. It is the drug of choice within the penicillins because it is better absorbed, following oral administration, than the other β-lactam antibiotics. However, amoxicillin usage in our health care system has drastically reduced as a result of many of the pathogens becoming resistant to it. As part of our ongoing study to screen local plants for their possible usefulness in chemotherapy of infectious diseases we assessed 16 plants used in herbal formulations in Ghana for their antibacterial properties and the effects of sub-inhibitory concentrations of extracts of these medicinal plants on the antibacterial activity of amoxicillin.

MATERIALS AND METHODS:

Plant material: The various plant parts as employed in folklore medicine in Ghana (**Table 1**) were harvested in October 2009 in the Ashanti Region of Ghana and authenticated at the Department of Pharmacognosy, Kwame Nkrumah University of Science and Technology, where herbarium specimens have been kept. The plant materials were separately air-dried for 15 days and milled into coarse powders using a Laboratory Mill Machine (Type 8, Christy & Norris, UK).

Extraction and Phytochemical screening: Five hundred grams each of the powdered plant materials were separately cold-macerated with methanol (Sigma-Aldrich, St. Louis, MO, USA) over 48 h. The extracts were filtered through Whatman filter paper #1 and concentrated under reduced pressure with a Rotary Evaporator. They were then dried in a hot air oven to constant weights at 40^{o}C. Portions of the dried extracts were screened for the presence of phytoconstituents using the procedures outlined by Wall [8], Harbon [9] and Sofowora [10].

Antibacterial Assay: The antibacterial activities of the plant extracts and amoxicillin (Sigma-Aldrich, St. Louis, MO, USA) were assessed against; *Staphylococcus aureus* (ATCC 25923), *Bacilus subtilis* (NCTC 10073), *Pseudomonas aeruginosa* (ATCC 27853), *Escherichia coli* (NCTC 25922) and *Salmonella typhi* (NCTC 8385). These test organisms were from the stock kept at the Microbiology Section of the Department of Pharmaceutics, KNUST, Kumasi, Ghana. Minimum inhibitory concentration (MIC) values of the extracts and amoxicillin were determined using the Kirby-Bauer agar diffusion method of antibacterial susceptibility testing [11]. A suspension of 24 h culture equivalent to a 0.5 McFarland standard was prepared in saline and spread onto pre-dried Mueller-Hinton agar plates. Four wells of 8 mm diameter were equidistantly bored in the agar and filled separately with 10 µl of 2, 4, 8, 16 and 32 mg/ml of the extracts which were reconstituted in 50 % methanol. The 50 % methanol was also tested as a control. Zones of growth-inhibition were read after 24 h incubation at 37^{o}C. The MICs were then calculated from semi-log plot of the values of concentration and mean zones of inhibition.

Amoxicillin Modulation Assay: In this assay, the minimum inhibitory concentrations of amoxicillin in the presence of sub-inhibitory concentration (10µg/ml) of the extracts were determined. The 10µg/ml solutions of the various extracts were used as vehicle for reconstituting the amoxicillin for use as detailed under antibacterial activity assay.

TABLE 1: SOME MEDICINAL PLANTS USED IN FOLKLORE TREATMENT OF INFECTIONS IN GHANA

Plant	Parts used	Specimen number	Folklore formulation
Anthoclesta nobilis	bark	FP/076/09	Decoction, tincture
Bambusa arundinacea	leaf	FP/077/09	Decoction
Bidens pilosa	whole plant	FP/078/09	Decoction, poultice
Croton membranaceus	root	FP/079/09	Tincture, decoction
Cryptolepis sanguinolenta	root	FP/080/09	Decoction, tincture
Elais guineensis	leaf	FP/081/09	Decoction
Hibiscus sabdariffa	calyx	FP/082/09	Decoction, concoction
Jatropha curcas	leaf	FP/083/09	Decoction, concoction
	root	FP/084/09	Decoction, poultice
Mallotus oppositifolius	leaf	FP/085/09	Decoction
Momordicha charantia	whole plant	FP/086/09	Decoction
Morinda lucida	bark	FP/087/09	Tincture, concoction, decoction
Moringa oleifera	root	FP/088/09	Powder, poultice,
Plumbago zeylanica	root	FP/089/09	Decoction, tincture
Psidium guajava	leaf	FP/090/09	Decoction, concoction
Spathodia campanulata	bark	FP/091/09	Decoction, poultice
Theobroma cacao	leaf	FP/092/09	Decoction
	seed	FP/093/09	Poultice

RESULTS AND DISCUSSION: Out of the 18 plant extracts tested 11 (61.1 %) exhibited significant antimicrobial activity against at least one of the five test bacteria with MICs below 20 mg/ml (**Table 2**). Extracts of *T. cacao* leaf and root, *J. curcas leaf* and *B. pilosa* were not active at the concentrations tested in this study. Extracts of *C. sanguinolenta*, *A. nobilis*, *H. Sabdariffa*, *P. guajava* and *P. zeylanica* showed broad spectrum antibacterial activity and the highest activities were exhibited by *C. sanguinolenta* root; *A. nobilis* stem bark and *H. sabdariffa* calyx. The presence of phytochemical compounds such as tannins, flavonoids, alkaloids, glycosides, anthraquinones and terpenoids in the extracts (**Table 3**) could account for their observed antibacterial activities. Plants have a long history of use in traditional medicine and have been the source of several promising novel antimicrobial agents which are not simply based on existing antibiotic templates [3, 4, 5].

These phytoconstituents may possess new, independent and different mechanisms of action from the existing antibiotics and may serve as leads for the development of new antimicrobials which will withstand the problem of bacterial cross-resistance development often encountered among many of the currently employed antibiotics such as the penicillins and cephalosporins. The MIC values obtained for amoxicillin were far lower than those of the extracts attesting to its superiority over the crude plant extracts. Amoxicillin (and other members of the penicillins) kills susceptible bacteria by specifically inhibiting the transpeptidase that catalyzes the cross-linking of peptidoglycan strands, the final step in cell wall biosynthesis [11].

Bacteria become resistant to amoxicillin when they produce β-lactamases to degrade the lactam ring which is necessary for the antibacterial activity of the penicillins. They may also produce modified transpeptidase enzymes with very low affinity for penicillin binding. Amoxicillin-resistant bacteria therefore survive, tolerate and replicate in the presence of the usual dose of the antibiotic and may be inhibited when the dose of the antibiotic is significantly increased. Any agent that reduces the MIC of amoxicillin may be said to have modified any of these mechanisms to allow the drug to act efficiently against the pathogen.

TABLE 2: MINIMUM INHIBITORY CONCENTRATIONS OF THE PLANT EXTRACTS

Plants/drug	Minimum Inhibitory Concentration (mg/ml)					
	Part	Ec	Sal	Pa	Sta	Bs
Amoxicillin		0.46±0.1[a]	4.7±0.2[a]	640±1.4[a]	0.29±0.1[a]	0.26±0.1[a]
Anthoclesta nobilis	bark	0.4±0.01	2.0±0.04	0.4±0.02	0.4±0.01	0.3±0.02
Bambusa aurambincea	leaf	>20	>20	-	>20	>20
Bidens pilosa	w. herb	-	-	-	-	-
Croton membranaceus	root	>20	13±0.04	16±0.3	>20	>20
Cryptolepis sanguinolenta	root	60±0.05[a]	0.3±0.02	40±0.3[a]	80±0.1[a]	10±0.01[a]
Elais guineensis	leaf	-	-	-	>20	>20
Hibiscus sabdariffa	calyx	4±0.06	2±0.04	-	2±0.03	2±0.1
Jatropha curcas	leaf	-	-	-	-	-
	root	2±0.01	8±0.04	-	>20	>20
Mallotus oppositifolius	leaf	-	-	6.5±0.01	11±0.03	11±0.04
Momordica charantia	w. herb	3±0.02	>20	-	>20	11±0.01
Morinda lucida	bark	9±0.02	8±0.04	10±0.04	12±0.06	12±0.01
Moringa oleifera	root	-	-	-	>20	>20
Plumbago zeylanica	root	16±0.01	2±0.01	16±0.03	4±0.01	12±0.02
Psidium guajava	leaf	4±0.01	10±0.02	9±0.02	2±0.02	10±0.03
Spathodia campanulata	bark	>20	19±0.04	15±0.04	>20	>20
Theobroma cacao	leaf	-	-	-	-	-
	seed	**-**	**-**	**-**	**-**	**-**

Key: [a] = values are in µg/ml, w. herb = whole herb, Ec = *Escherichia coli*, Sal = *Salmonella typhi,* Pa = *Pseudomonas aeruginosa*, Sta = *Staphylococcus aureus*, Bs = *Bacillus subtilis*, and - = no activity observed

TABLE 3: PHYTOCONSTITUENTS PRESENT IN THE PLANT EXTRACTS

Constituents	Plant species
Saponins	*A. nobilis, B. pilosa, C. membranaceus, C. sanguinolenta, H. sabdariffa, J. caurcas, M. oppositifolius, M. charantia, M. lucida, M. oleifera, P. zeylanica, P. guajava, S. campanulata, T. cacao*
Cardiac glycosides	*B. pilosa, M. oppositifolius, P. guajava*
Cyanogenic glycosides	*B. arundinacea, J. caurcas*
Flavonoids	*A. nobilis, B. arundinacea, B. pilosa, C. membranaceus, C. sanguinolenta, E. guineensis, H. sabdariffa, J. caurcas, M. oppositifolius, M. lucida, M. oleifera, P. zeylanica, P. guajava, S. campanulata, T. cacao.*
Alkaloids	*A. nobilis, B. arundinacea, B. pilosa, C. membranaceus, C. sanguinolenta, E. guineensis, H. sabdariffa, J. caurcas, M. charantia, M. lucida, M. oleifera, S. campanulata, T. cacao.*
Terpenoids	*A. nobilis, C. membranaceus, C. sanguinolenta, E. guineensis, H. sabdariffa, J. caurcas, M. oppositifolius, M. charantia, M. lucida, M. oleifera, P. zeylanica, P. guajava, S. campanulata, T. cacao.*
Tannins	*A. nobilis, B. pilosa, C. membranaceus, C. sanguinolenta, E. guineensis, H. sabdariffa, J. caurcas, M. oppositifolius, M. lucida, M. oleifera, P. zeylanica, P. guajava, S. campanulata, T. cacao.*
Anthraquinones	*H. sabdariffa, M. lucida, M. oleifera*

The antibacterial activity of amoxicillin against *Staph. aureus* was significantly ($p<0.05$) enhanced by *M. oppositifolius, B. pilosa, M. lucida, C. membranaceus* and *J. curcas* extracts. *B. subtilis* also became significantly susceptible to amoxicillin in the presence of sub-inhibitory concentration of *B. pilosa, H. sabdariffa*, *M. oppositifolius, M, charantia, A. nobilis, C. sanguinolenta* and *M. oleifera* extracts. *S. campanulata, M. lucida, M. oleifera* and *J curcas* leaf extracts also significantly reduced the MIC of amoxicillin against *E. coli* while *S. typhi* susceptibility was enhanced by the presence of *A. nobilis*, *M. charantia* and *J. curcas* extracts. However, none of the extracts improved the susceptibility of *P. aeruginosa* against amoxicillin (**Fig. 1**). Even though the presence of *T. cacao, E. guineensis, P. guajava* and *B. arundinacea* extracts did not exhibit any modification of amoxicillin activity against any of the test bacteria, in our earlier study these plants (in addition to others) potentiated tetracycline activity [12].

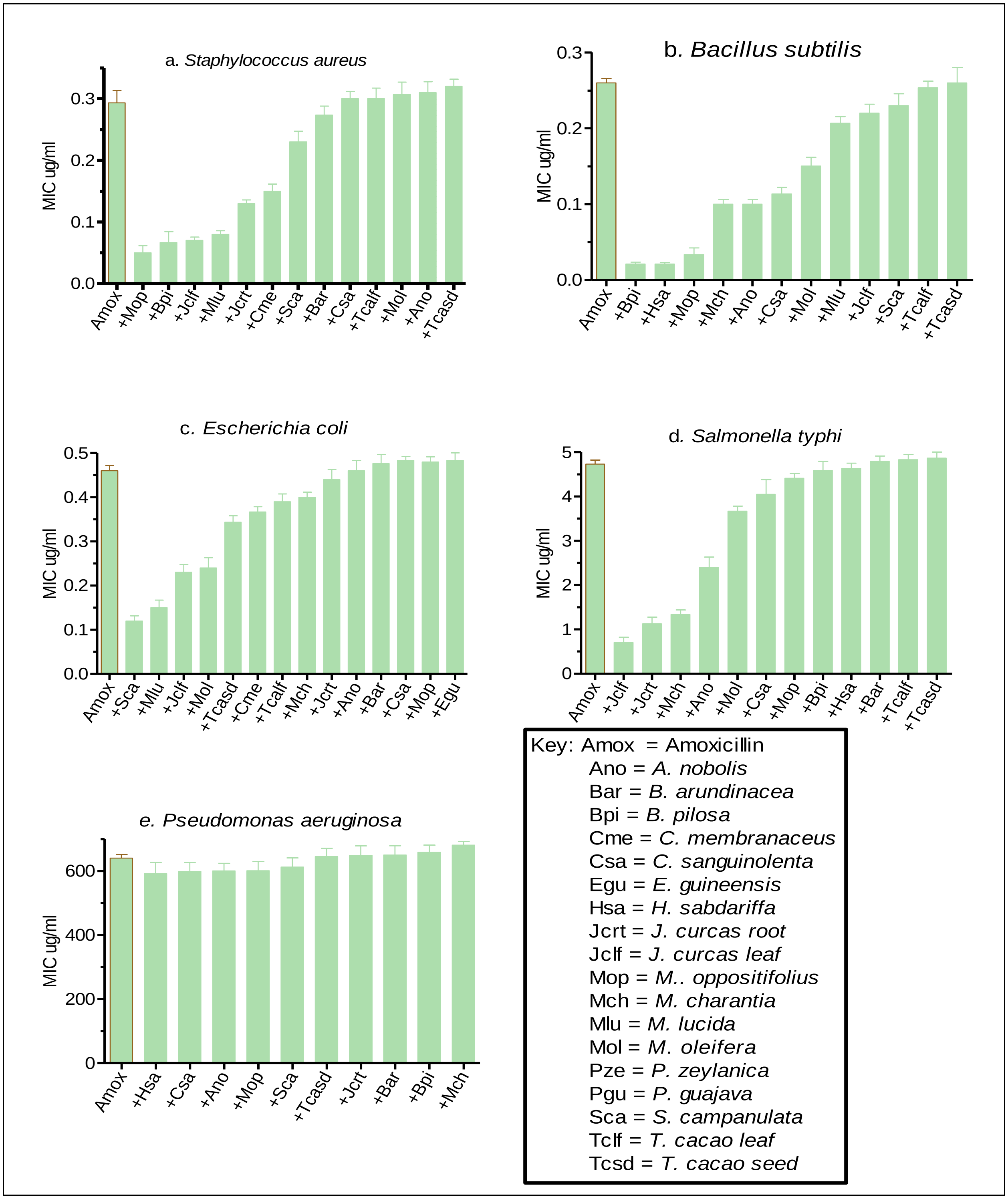

FIG. 1: MICS OF AMOXICILLIN ONLY AND AMOXICILLIN IN THE PRESENCE OF SUB-INHIBITORY CONCENTRATIONS OF PLANT EXTRACTS

Interestingly, *J. curcas*, *B. pilosa* and *M. oleifera* extracts did not exhibit significant antibacterial activity but their presence enhanced amoxicillin activity against at least two of the test organisms: *B. pilosa* (*Staph. aureus* and *B. subtilis), M. oleifera* (*B. subtilis* and *E. coli*) and *J. curcas* (*Staph. aureus, E. coli* and *S. typhi*). These findings are in line with earlier reports of plant constituents including alkaloids [13], flavonoids and coumarins [14], tannins and saponins [15], terpenoids and steroids [16] exhibiting bacterial resistance modulation properties. These plants therefore appear to be a rich source of leads for chemotherapeutic drug development.

Combination chemotherapy is preferred in the management of resistant bacterial infections. A typical example is seen with Augmentin (from GlaxoSmithKline) where clavulanic acid inhibits the function of β-lactamases produced by resistant bacteria and thus prevents destruction of the amoxicillin. We also recently reported the antibiotic-modifying properties of friedelin (isolated from *Paullinia pinnata*) and extracts of *Corynanthe pachyceras* [17, 18]. These plants may provide cheaper antibiotic modulation adjuvants for amoxicillin formulations which may be more affordable for treatment of resistant bacterial infections encountered especially among HIV/AIDS patients.

CONCLUSION: It has therefore been demonstrated that some plant extracts even though exhibit no antimicrobial activity can potentiate the activity of some antibiotics. Hence, plant sources of antibacterials must not be overlooked as their antibacterial activity and enhancement of antibiotic activity are in many cases appreciable.

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