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Drug interaction studies of *Ximenia americana* and *Pavetta crassipes* methanol extract with standard antibiotics

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Abstract
The therapeutic efficacy of single or multicomponent herbs is thought to reside in synergistic interactions between the bioactive constituents. The methanol extracts of *X. americana* and *P. crassipes* were initially screened against Gram positive and negative organisms as well as against *Mycobacterium tuberculosis* H37Rv but the antibacterial activities were not potent enough to warrant further explorations as stand-alone antibacterials. Using the agar diffusion method, standard antibiotics were screened for drug susceptibility against *Staphylococcus aureus* (ATCC 25823), *Escherichia coli* (ATCC 11560) and *Pseudomonas aeruginosa* (ATCC 10662). The antimicrobial activities were then screened against the same organisms in the presence of the extracts at a final concentration of 5 mg/ml in order to determine the influence on the inhibitory activities by measuring changes in the zone diameters. The study showed that the antibacterial interaction with *P. crassipes* extract was more prominent with the penicillins and tetracycline against *S. aureus* and *P. aeruginosa* while *X. americana* had prominent interaction with the same antibiotics in addition to cotrimoxazole and the aminoglycosides, streptomycin and gentamicin. The interaction between the extracts and some of the antibiotics could be further explored for synergism especially with the Gram negative organisms.

Keywords: Drug interaction; Antibiotics; *P. crassipes; X. Americana; S. aureus; E. coli; P. aeruginosa*

INTRODUCTION

The concept of synergism in phytomedicines arose out of difficulties experienced in isolating bioactive compounds from herbal products or in adducing clinical effects to single constituents in phytomedicines (Williamson, 2001). The therapeutic efficacy of single or multicomponent herbs is thought to reside in synergistic interactions between the bioactive constituents. Often, long-lasting drug administration is required to achieve the desired clinical effect with reduction in severity of symptoms (Heinrich *et al*., 2012). This mode of drug administration can be likened to treatment of conditions such as depression, cancer, HIV in orthodox medicine where clinical effects become evident after a number of weeks (Williamson, 2001).

Another identical characteristic of phytomedicines and orthodox medicine is the need for drug combinations in treating chronic conditions. According to Heinrich *et al.* (2012), many phytomedicines are yet to have their mechanisms of action elucidated. The article reports that there are examples of

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total herb extracts showing better effects than equivalent doses of isolated compounds. Justification for the biological effect observed has been attributed to synergy, enhanced bioavailability, cumulative effects or simply the additive properties of the constituents.

Overall, synergy is considered to be positive with the low doses used being seen as an advantage. Some ingredients which form part of herbal drug combinations have been investigated and reported to enhance bioavailability of other constituents. For instance, addition of black pepper, *Piper longum* which contains the alkaloid piperine to an anti-asthma recipe has been reported to increase the bioavailability of the mixture (Johri and Zutshi, 1992; Fidai *et al*., 1997; Khajuria *et al*., 1998) and also the addition of clavulanic acid to amoxicillin used to increase susceptibility to β-lactamase producing organisms in conventional antimicrobial chemotherapy (Flores *et al*., 2005). Bapela *et al.* (2006) investigated the activity of a combination of 7-methyljuglone, a naphthoquinone and first-line drugs, isoniazid and rifampicin in which they reported a four to six-fold reduction in the MICs of both drugs suggestive of synergy. In some other cases, negative or unwanted effects have also been observed to interfere with absorption of active constituents leading to decreased therapeutic concentrations. Drug interaction studies conducted on St John's Wort, *Hypericum perforatum (*herbal remedy) and oral contraceptives reportedly induced increased enzyme activity in some cases while Will-Shahab *et al*. (2009) in their study found that St John's wort did not alter the pharmacokinetics of a low-dose oral contraceptive (Wang *et al*., 2001; Hall *et al*., 2003; Pfrunder, 2003). The initial drug susceptibility study by Odumosu (2012) on the methanol extracts of both plants against *M. tuberculosis* H_{37}Rv ATCC 27294 (virulent strain) showed no inhibition at MIC > 128 µg/ml so this study was conducted to determine the interaction of the methanol extract of *X. americana* and *P. crassipes* with standard antibiotics for potential synergy owing to the fact that opportunistic organisms have been found to co-exist with *M. tuberculosis* infection.

EXPERIMENTAL

Materials. These include: Iso-Sensitest agar (ISA); broth powder (CM 0473) (Oxoid, U.K). Hexane, dichloromethane, methanol and dimethyl sulfoxide were purchased from Sigma Aldrich, U.K while Mastring - M14 antibiotic discs were purchased from Mast Diagnostics, Merseyside, UK. Class II microbiological safety cabinet was supplied by Atlas labs, U.K.

Bacterial organisms. Control organisms, representative of bacteria, were chosen according to British Society for Antimicrobial Chemotherapy (BSAC) guidelines. The typed organisms used in the antimicrobial screening were *Staphylococcus aureus* (ATCC 25823), *Escherichia coli* (ATCC 11560) and *Pseudomonas aeruginosa* (ATCC 10662).

Sample preparation. Dried powdered *X. americana* (root) and *P. crassipes* (leaf) plant material were sequentially extracted with hexane, dichloromethane, methanol and water to obtain the various extracts. The methanol extract was used to conduct the study. Reagents and media were prepared according to BSAC (bsac.org.uk/susceptibility) guidelines and manufacturer's instructions.

Drug interaction assay using Mastring S antibiotic strips. Mastring S-14 (antibiotic containing strip) was placed on agar plates without plant extracts (control plates) and duplicates incorporated with plant extracts to achieve a final concentration of 50, 100, 5000 and 10,000µg/mL. The assays were conducted as four different experiments. The plates were inoculated with *S. aureus*, *E. coli* and *P. aeruginosa* prepared according to BSAC guidelines. Plates containing solvent used in

sample solution and negative control plates were added to validate the results. Inhibition zones were measured and percentage differences calculated in order to determine which antibiotics showed some interaction in the preliminary assessment. Tests were carried out in triplicate.

RESULTS AND DISCUSSION

The combination of *X. americana* and *P. crassipes* is used in the treatment of respiratory infections such as TB in herbal medicine. Screening of the methanol solvent fractions against *M. tuberculosis* H37Rv (virulent strain) in previous studies showed little inhibitory activities so the drug interaction study was conducted to determine potential synergistic properties with other antibiotics. The antibiotic strip (Mastring S-14) contained the following antibiotics listed in Table 1. The data obtained from the *in vitro* drug interaction studies using *S. aureus, P. aeruginosa* and *E. coli* are shown in Figures 1-3. Several antibiotic compounds (Mastring S antibiotic strip) were tested against organisms, *S. aureus* and *P. aeruginosa, E. coli* by agar containing the plant extracts.

Table 1: Mastring S M-14 antibiotic discs content with code

Code	Antibiotic	Colour	Disc content (µg)
AP	ampicillin	Grey	10
KF	cephalothin	Primrose	5
CO	colistin SO_4	White	25
GM	gentamicin	Salmon	10
S	streptomycin	White	10
ST	sulphatriad	Mauve	200
T	tetracycline	Brown	25
TS	cotrimoxazole	White	25

Table 2: Summary of *in vitro* interaction studies of antibacterials with *P. crassipes* methanol extract (5 mg/mL final conc.)

Organism	Increased zone of inhibition	Inhibition cancelled	Inactive antibiotic active with extract	Indifference
S. aureus	ampicillin, cephalothin tetracycline	streptomycin		colistin SO_4, sulphatriad cotrimoxazole
P. aeruginosa	tetracycline	colistinSO_4, streptomycin sulphatriad	ampicillin, cephalothin	Gentamicin Cotrimoxazole
E. coli		streptomycin tetracycline	cephalothin	ampicillin, colistin SO_4, gentamicin, sulphatriad, cotrimoxazole

Table 3: Summary of *in vitro* interaction studies of antibacterials with *X. americana* methanol extract (5 mg/mL final conc.)

Organism	Increased inhibition zone	Inhibition cancelled	Inactive antibiotic active with extract	Indifference
S. aureus	ampicillin, cephalothin gentamicin, tetracycline, cotrimoxazole, streptomycin		colistin SO_4, sulphatriad	
P. aeruginosa		sulphatriad, tetracycline, cotrimoxazole		ampicillin, cephalothin, streptomycin
E.coli	gentamicin, cotrimoxazole	streptomycin		ampicillin, cephalothin, sulphatriad, tetracycline

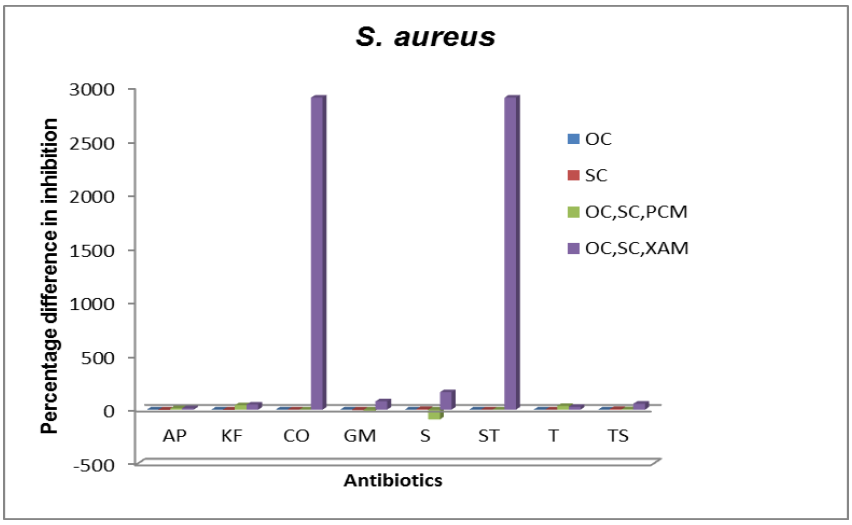

Figure 1: Chart showing percentage differences in zone diameter of inhibition between antibiotics alone and in combination with extracts, PCM and XAM. Extracts were incorporated into agar to give final concentration of 5 mg/mL. Values recorded are average of three replicates (n = 3). *SE values were ≤ 0.57

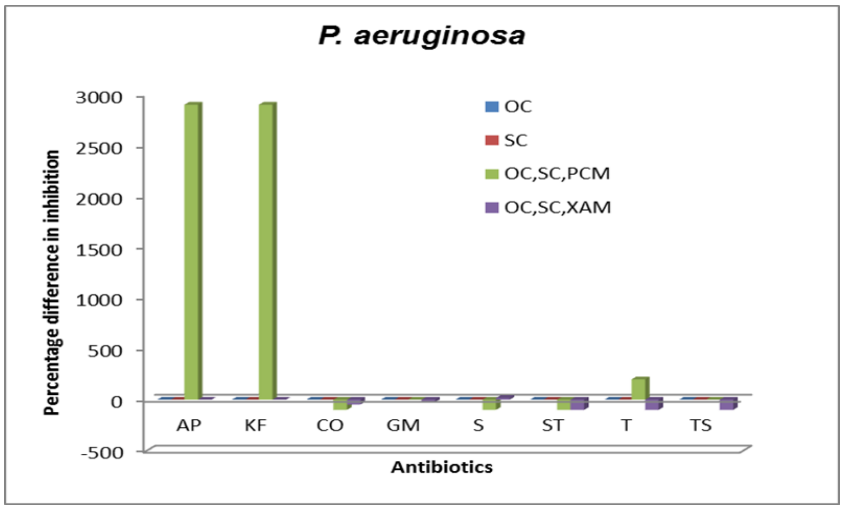

Figure 2: Chart showing percentage differences in zone diameter of inhibition between antibiotics alone and in combination with extracts, PCM and XAM.). *SE values were 0.0

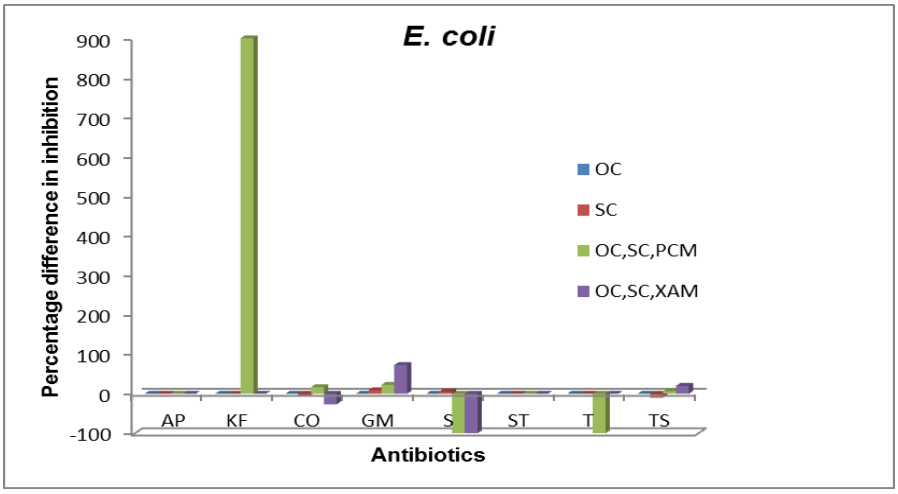

Figure 3: Chart showing percentage differences in zone diameter of inhibition between antibiotics alone and in combination with extracts, PCM and XAM. *SE values were ≤ 0.66

Legend: OC – antibiotic alone with organism; SC - antibiotic with organism (contains solvent used in dissolving extract); PCM - methanol extract of *P. crassipes;* XAM - methanol extract of *X. americana;* Increased zone diameter of inhibition - (change of ≥ 3mm); Decreased zone diameter of inhibition - (change of ≥ 3mm)

Extracts were incorporated to a final concentration of 50, 100, 5000 and 10,000 µg/mL. There were no changes in zones of inhibition with 50 and 100 µg/mL but with a final concentration of 5000 µg/mL, some antibiotics exhibited additive or synergistic interactions. Interestingly, there was no growth with 10000 µg/mL of extracts incorporated into agar which could possibly result from toxicity leading to cell death or potency of the active constituents. The zones of inhibition measured for antibiotics alone and with extract (PCM and XAM) incorporated to give final concentration of 5000 µg/ mL were used to calculate the percentage difference in inhibition zone diameter. A difference of ≥ 3mm was considered as inhibitory interaction.

XAM exhibited moderate activity with streptomycin against *S. aureus* but the inhibitory effect was greater with colistin sulphate (CO) and sulphatriad (ST). Initial screening of CO and ST showed no inhibitory activity but the presence of XAM enhanced its activity by approximately 3,000 %. In comparison, PCM did not show appreciable activity against *S. aureus* (Figure 1).

In clinical settings, sulphatriad (an anti-metabolite) which consists of a mixture of suphathiazole, sulphadiazine and sulphamerazine is used as a combination product to reduce the risk of crystalluria associated with sulphonamide use. However, it is not commonly used in clinical practice due to the associated kidney damage from accumulation of crystals in urine (Mitscher *et al.*, 2008). The factor in favour of its continued use in certain conditions is the relative cheapness compared to newer antibiotics. Chemically, colistin sulphate is a cyclic polypeptide antibiotic from *Bacillus colistinus* and consists of polymixins E1 and E2 which act as a detergent to disrupt bacterial cell membrane. It has been found useful for treating infections with *P. aeruginosa* but like the sulphonamides, it has been largely abandoned in the clinical setting with the development of newer penicillins and cephalosporins. Some investigators (Knox *et al*., 2011; Wishart *et al*., 2008; Wishart *et al*., 2006) reported the usefulness of colistin in treating multi-drug resistant pulmonary infection in cystic fibrosis patients.

PCM had strong positive effect on antibiotics, ampicillin and cephalothin against *P. aeruginosa* and *E. coli* (cephalothin only) and to a lesser extent on tetracycline, ampicillin and cephalosporin (Figures 2 & 3). Decrease in inhibition zones were observed with streptomycin against *E. coli and S. aureus* in the presence of PCM unlike XAM which had a positive effect (Figures 1, 2 & 3). Some of the antibiotic inhibitory activities were cancelled due to some antagonism while some others showed no changes. The *in vitro* interaction studies of the antibiotics with *P. crassipes* methanol extract (5 mg/ml) and *X. americana* methanol extract (5mg/ml) are summarized in Tables 2 and 3.

Conclusion: The antibacterial interaction with *P. crassipes* extract was more prominent with the penicillins and tetracycline against *S. aureus* and *P. aeruginosa* while *X. americana* had prominent interaction with the same antibiotics in addition to cotrimoxazole and the aminoglycosides, streptomycin and gentamicin.

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