HYGEIA
JOURNAL FOR DRUGS AND MEDICINES
ISSN 2229 3590 (on line) ISSN 0975 6221 (print)

The effects of *Acanthospermum hispidum* extract on the Antibacterial activity of Amoxicillin and Ciprofloxacin

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Article history: Received: 1December, 2010, revised: 12 January, 2011, accepted: 20 February, 2011, Available online: 14 April 2011

Abstract

The effect of the sub-inhibitory concentrations of *Acanthospermum hispidum* extract on the activities of amoxicillin and ciprofloxacin was assessed by the Kirby-Bauer disc diffusion method. The extract demonstrated antibacterial activity against *Staphylococcus aureus, Klebsiella pneumoniae, Bacillus subtilis, Salmonella thyphi, Proteus vulgaris* and *Pseudomonas aeruginosa* with MICs ranging between 11 and 53 mg/ml. A 5 mg/ml extract enhanced significantly ($p<0.05$) ciprofloxacin activity (up to 38 folds) against all the test bacteria except *Ps. aeruginosa*. Amoxicillin activity was also potentiated significantly ($p<0.05$) against *Staph. aureus* (9 fold) and *B. Subtilis* (12 fold). *A. hispidum* appears to contain phytoconstituents that may be useful adjuvant for antibiotic formulations.

Key words: Sub-inhibitory concentration, antibacterial activity, amoxicillin, ciprofloxacin, *A. hispidum.*

1. Introduction

Antibiotics, once considered the universal answer to infectious disease, are now known to have a limited effective life span. Disease causing microorganisms that were once thought to have been controlled by antibiotics are returning in new forms resistant to antibiotic therapy [1]. Incidents of epidemics due to such drug resistant microorganisms are now a common global problem posing enormous public health concerns. While chemical modifications could be significant in antibiotic resistance, exclusion from the cell of unaltered antibiotic represents the primary strategy in denying the antibiotic access to its targets and this is believed to enhance resistance even in cases where modification is the main mechanism [2].The use of agents that do not kill pathogenic bacteria but modify them to produce a phenotype that is susceptible to an antibiotic could be an alternative approach to the treatment of infectious diseases. Such agents could render the pathogen susceptible to a previously ineffective antibiotic, and because the modifying agent applies little or no direct selective pressure, this concept could slow down or prevent the emergence of resistant genotypes [3].

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A similar approach can be used to target modifying enzymes and efflux systems.A number of *in vitro* studies have shown plant extracts to significantly reduce the minimum inhibitory concentrations (MICs) of antibiotics against some resistant pathogenic bacteria [4], [5], [6], [7]. It is speculated that inhibition of drug efflux and alterations in membrane permeability could be responsible for the synergistic interactions between plant extracts and antibiotics [8]. The use of plant constituents in a bid to fight against the spread of antibiotic resistant pathogens is still an untapped resource [9].

Acanthospermum hispidum(DC), (family Compositae) is used in traditional medicine for the treatment of jaundice, malaria, stomachache, constipation, fever, [10] and viral infections [11]. The ethanolic extracts of the leaves and flowering tops have showed varying degrees of activity against a wide range of pathogenic bacteria [12]. We hereby report on the effect of this plant extract on the antibacterial activity of some antibiotics.

2. **Materials and methods**

2.1. **Plant material.**

The aerial parts of *Acanthospermum hispidum* were collected from the KNUST Campus, and identified in the Department of Pharmacognosy where a voucher specimen (FP/094/10) has been deposited. They were washed and sun dried for 5 days and then milled into coarse powder using a laboratory Mill Machine (Type 8, Christy & Norris, UK).

Three hundred and fifty grams of the powder was Soxhlet extracted using 70% ethanol and concentrated under reduced pressure using a Buchi Rotavapor R-114. The concentrate was evaporated to dryness at 40^{o}C in a hot air oven. The extract (38.7g; yield 11.0%) was stored in an airtight container at 4°C.

2.2. **Microorganisms used for the tests**

organisms used for the tests were: *Staphylococcus aureus* ATCC 25923 *Klebsiella pneumoniae* ATCC 31488, *Bacillus subtilis*, NCTC 10073, *Salmonella typhi* ATCC 19430, *Proteus vulgaris* NCTC 4635 *Pseudomonas aeruginosa* ATCC 27853. These were subcultures from of the stocks kept in the Pharmaceutical Microbiology Laboratory, KNUST, Kumasi.

2.3. **Antimicrobial activity determination**

The antimicrobial activity was determined using the Kirby-Bauer agar disc diffusion method [13].
25 ml Muller Hinton agar (Sigma-Aldrich, St Louis, MO, USA) plate was poured and allowed to set. 10 µl of the bacterial culture, diluted to 0.5 McFarland standards with saline, was spread over the surface using a spreader.
400 mg/ml solution of the extract was prepared in 50% methanol and serial dilutions were made to produce 300 mg/ml, 200 mg/ml and 100 mg/ml solutions.

Filter paper discs (6 mm) soaked with 30 µl of the various concentrations of the extracts as prepared above were placed at various marked positions. The test plate was allowed to stand for one hour and then incubated at 37^{o}C for 24 hours. The 50% methanol was also tested as a control.

The minimum inhibitory concentrations (MIC) of the extract against the various organisms were then calculated from semi-log plot of values of concentration and mean zones of inhibition.

3. **Antibiotic resistance modulation assay**

In the resistance modulation assay, the MICs of the antibiotics (amoxicillin and ciprofloxacin, Sigma) against the various organisms were determined by the agar disc diffusion method as above. The MICs of the antibiotics were re-determined by using a sub-inhibitory concentration of the extract (5mg/ml) as solvent for the preparation of the various concentrations of the antibiotics.

4. **Results**

The ethanolic extract of *A. hispidum* showed antimicrobial activity against all the test microorganisms (Table 1). *B. subtilis* was the most sensitive organism while *Ps. aeruginosa* was the least sensitive.

Table 1 Minimum Inhibitory Concentrations (MIC) of *A. hispidum* extract against organisms

Organisms	MIC (mg/ml)
S. aureus	44.7
B. subtilis	11.0
K. pneumonia	48.0
P. aeruginosa	52.5
P. vulgaris	39.0
S. typhi	39.0

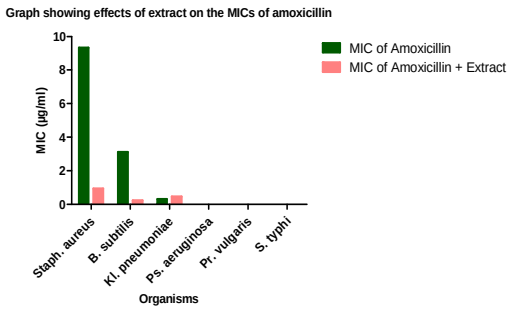

Figure-1

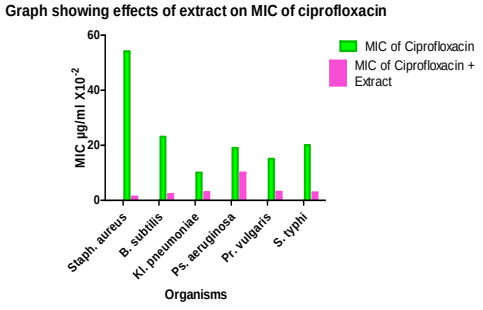

Figure-2

5. **Discussion**

Acanthospermum hispidum has been found to possess antimicrobial activity [12]. The crude ethanol extract of *A. hispidum* showed varying degrees of activities (table 1) against all the test organisms used. The plant extract was most active against *B subtilis* aqnd least active against *Ps aeruginosa.*

The extract demonstrated resistance modifying activity with both amoxicillin and ciprofloxacin.

Amoxicillin is a β-lactam antibiotic. Susceptibility to β-lactam antibiotics reflects the combined effects of binding to targets (penicillin-binding proteins [PBPs]), stability to β-lactamases, and, in Gram-negative bacteria, outer-membrane permeability. Similarly, resistance reflects a change in any of the three components [14].

Penicillin binding proteins (PBPs) are present in almost all bacteria, but they vary from species to species in number, size, amount, and affinity for β-lactam antibiotics, usually following taxonomic lines [14]. They are localized non-randomly on the outer face of the cytoplasmic membrane [15] and are anchored through short hydrophobic carboxy- or amino-terminal sequences; in Gram-negative bacteria, they are pseudoperiplasmic [16].
Amoxicillin combined with the plant extract produced a significant resistance modifying activity ($p<0.05$) against *Staph. aureus* (9 fold increase in activity) and *B. subtilis* (12 fold increase in activity), both Gram positive bacteria (Fig. 1). There was a slight reduction in the activity of amoxicillin against *Kl pneumoniae* (Gram negative bacteria). Amoxicillin showed no activity against *Ps. aeruginosa*, *Pr. vulgaris,* and *S. typhi* (Gram negative organisms) at the concentrations used both in the presence and absence of the extract.
Traditionally, the major mechanism of β-lactam resistance has involved β-lactamases, particularly plasmid-mediated β-lactamases. In Gram-negative bacteria, β-lactamases are periplasmic and act in combination with altered outer membrane permeability. In Gram-positive bacteria, they are exocellular, although they are probably associated with the cell wall through electrostatic interactions [17].

The major enzymatic activities associated with PBPs are peptidoglycan transpeptidase, which is believed to be essential, and DD-carboxypeptidase, which is believed to be dispensable. In a given organism, there are two to four essential PBPs and, thus, potentially multiple β-lactam targets. Altered PBPs associated with β-lactam resistance are more commonly found in Gram-positive than in Gram-negative bacteria [14].

This suggests that the resistance modifying activity exhibited by the extract on amoxicillin may be due to constituents that act on the cell wall, which probably, inhibit β-lactamase activity, makes the cell wall more permeable or enhances the interaction between amoxicillin and target sites.

The primary target of all the fluoroquinolones including ciprofloxacin is DNA gyrase (topoisomerase II), [18].

Two mechanisms of quinolone resistance have been described; alterations in the targets of quinolone and decreased accumulation due to impermeability of the membrane and/or over expression of efflux pump systems. Mobile elements have also been described carrying the *gnr* gene which confers resistance to quinolones [19].

A combination involving Ciprofloxacin with the sub-inhibitory concentrations (5mg/ml) of *A. hispidum* extract produced MICs that are significantly lower than the standard drugs alone ($p<0.05$) against all the bacteria used (Fig. 2). There was a high increase in activity (38 fold decrease in MIC) against *Staph. aureus* and *B. subtilis* (10 fold). There were increases in activity against *S. typhi*, *Pr. vulgaris*, and *Kl. pneumoniae* (7 fold, 5 fold, 3 fold respectively).

There was only a marginal decrease in activity (< 2 fold) against *Ps. aeruginosa.* As in the case of amoxicillin the resistance modifying activity is more pronounced with Gram positive organisms than the Gram negatives.

The results of the study pre-suppose that the effects of the extracts are on the cell wall peptidoglycan permeability. Increasing permeability of the cell wall enhanced the activity of both drugs against Gram positive organisms (which contain 50-90% peptidiglycan) because of better interaction between antibiotic and organism. For Gram negative organisms which have much less peptidoglycan (5-10%) the effects were minimal [20].

These results suggest that, there is possibly some phytochemical components in the ethanol extract of *A. hispidum* which affected one or more of the resistance mechanisms of these organisms probably the efflux mechanisms or increased the binding of the antibiotic to target cells and as such, increased the efficacy of the drugs. This is similar to the observations made by [5].

6. **Conclusion**

Sub-inhibitory concentrations of *A. hispidum* (5mg/ml) enhanced the activity of amoxicillin against *Staph. aureus* and *B. subtilis* but reduced slightly the activity against *Kl. pneumoniae*. Combining ciprofloxacin with the sub-inhibitory concentrations (5mg/ml) of *A. hispidum* extract modulated the resistance of all the organisms to ciprofloxacin. The resistance modulatory activity of the extracts on amoxicillin and ciprofloxacin is more pronounced with Gram positive organisms than Gram negative organisms.

The findings show that it is possible to find a phytoconstituent in *A. hispidum*, which in combination with ciprofloxacin and amoxicillin will produce a regimen that is capable of managing infections due to organisms that are resistant especially to ciprofloxacin.

Acknowledgements

The authors are very grateful to the government and people of Ghana for providing funds for the study.

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