

RESEARCH PAPER
**ANTIMICROBIAL AND MODULATION EFFECTS OF
SELECTED GHANAIAN MEDICINAL PLANTS**

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ABSTRACT

*The alarming rise in the incidences of multidrug-resistant microorganisms and the decline in new antibiotic discovery make the search for new antimicrobial agents or efforts at restoring the activity of older antibiotics to which the microbes have developed resistance very necessary. The aim of the present study is to investigate the antimicrobial and modulation effects of the 70% ethanol extracts of *Lannea schimperi*, *Commelina nudiflora* and *Piliostigma reticulatum* against susceptible strains of microorganisms. Using the broth microdilution method, the minimum inhibitory concentrations (MICs) of the extracts were determined. The checkerboard assay was used to determine the modulation effects when sub-inhibitory concentrations of plant extracts were combined with the standard antibiotics. All three plants extracts possessed weak antimicrobial effects. For the modulation experiments, fifteen of the twenty-seven combinatorial cases yielded biologically significant effects. The ethanol extracts of the three plants studied here are good modulators as they reduced the MIC of ciprofloxacin and ketoconazole by factors that are comparable to that of reserpine. However the exact compounds and their exact mechanism of modulation require further investigation.*

Keywords: *Anti-infective, modulation, *Lannea schimperi*, *Commelina nudiflora*, *Piliostigma reticulatum*, ethnomedicine*

INTRODUCTION

Antimicrobial agents since their introduction have greatly reduced illness and death from infectious diseases. However in the last few decades, the spread of new diseases, the re-emergence of diseases thought of as under control, the alarming trend of antimicrobial resis-

tance and the decline in new antibiotic discovery make the search for new antimicrobial agents necessary. The risk of increased economic and social costs and death make antimicrobial resistance a threat to global public health. If allowed to spread, this phenomenon would undermine infectious disease control and

reverse the gains made so far (WHO, 2014).

Multidrug-resistant (MDR) microorganisms, namely, methicillin-resistant *Staphylococcus aureus*, vancomycin resistant enterococci, extended-spectrum cephalosporin-resistant *Escherichia coli* and *Klebsiella* species and MDR *Pseudomonas aeruginosa* most frequently cause life-threatening diseases in humans (Giske *et al.*, 2008; Talbot *et al.*, 2006). The current reports of resistance, means that treatment for severe infections, for which these organisms are implicated, may need to be initiated with different treatment options. The challenge though is that fewer treatment options are available and are costly for people living in low-income countries. To restore the activity of cheaper antibiotics that MDR bacteria have made inactive, research has been devoted to finding new agents from natural sources that can modulate bacterial resistance (Kovač *et al.*, 2015). An agent is said to possess a modulatory effect on an antibiotic if it is able to reduce its MIC by certain factors (Dickson *et al.*, 2006).

Medicinal plants serve as potential sources of anti-infective and resistance modifying agents. *Commelina nudiflora* L. (Commelinaceae), *Lannea schimperi* (Hochst. Ex A. Rich.) Engl. (Anacardiaceae) and *Piliostigma reticulatum* (DC) Hochst. (leguminosae) are medicinal plants which find use in traditional medicine in the treatment and management of infectious diseases (Contributors, 2015 and Kisangau *et al.*, 2007). Previous studies on *P. reticulatum* has shown antimicrobial (Awe and Omojasola, 2009; Babajide, *et al.*, 2008; Zerbo *et al.*, 2013) and trypanocidal (Atawodi *et al.*, 2004) activities. *C. nudiflora* has also been shown to exhibit antimicrobial activities (Kuppusamy *et al.*, 2015). In addition, *L. schimperi* has been shown to possess antibacterial (Haule *et al.*, 2012) and antifungal (Kisangau *et al.*, 2009) activities.

These plants are being investigated here for their antimicrobial and resistance modulation effects using antibiotic-susceptible strains of

microorganisms.

METHODS

Plant collection and authentication

The stem bark of *Piliostigma reticulatum* and the fresh leaves of *Commelina nudiflora* were collected from the Kwame Nkrumah University of Science and Technology (KNUST) Botanical garden. The stem bark of *Lannea schimperi* was collected from Kwahu Asakraka in the Eastern region. All three plants were collected in August 2014. Botanical identities were confirmed at the Department of Herbal Medicine herbarium, KNUST. Voucher specimen of *C. nudiflora* (KNUST/HM1/2015/L009), *L. schimperi* (KNUST/HM1/2015/S006) and *P. reticulatum* (KNUST/HM1/2015/S032) have been deposited in the same department.

Microorganisms used

Nine microorganisms, including four Gram-positive bacteria (*Enterococcus faecalis* ATCC 29212, *Streptococcus pyogenes* clinical strain, *Staphylococcus aureus* ATCC 25923 and *Bacillus subtilis* NCTC 10073), four Gram-negative bacteria (*Salmonella typhi* NCTC 6017, *Escherichia coli* NCTC 9002, *Pseudomonas aeruginosa* ATCC 27853 and *Klebsiella pneumonia* clinical strain) and one fungi (*Candida albicans* clinical strain) were used for the antimicrobial and modulation tests.

Extraction of plant materials

Fifty grams of the air-dried plant materials were soxhlet extracted using 500 ml of 70 % ethanol for 12 h. The resulting extracts were reduced to dryness on a rotary evaporator. The percentage yields for the *L. schimperi*, *C. nudiflora* and *P. reticulatum* were 5.53, 6.20 and 5.70 % w/w respectively. A 20 mg/mL stock solution was prepared by dissolving 0.1 g of the ethanolic extracts in 5 ml of DMSO.

Phytochemical screening

The presence of phenolics, saponins, steroids, reducing sugars, flavonoids, alkaloids and triterpenes were detected using simple qualitative phytochemical methods (Evans, 2009).

Antimicrobial and modulation assays

Minimal inhibitory concentrations (MICs) of the plant extracts and the standard drugs (ciprofloxacin and ketoconazole) were determined using the micro-well broth dilution methods. Six dilutions were made from the stock solution of the plant extracts by two-fold serial dilutions in sterile distilled water. The resultant concentrations in the wells were between 9.09 mg/ml and 0.14 mg/ml. Inoculums of microorganisms were prepared from 12 h broth cultures and serial dilutions were made to achieve a suspension of approximately 10⁵ CFU/ml.

The wells were prepared by dispensing 100 µl of double strength nutrient broth and 100 µL each of the test samples together with 20 µl of the inoculum. After 24 h incubation at 37 °C, optical densities in each well were read using the Synergy HT microplate reader (Bio-Tek Instruments, USA), at 600 nm. Percentage growth was calculated according to the following equation:

$$\% \text{ Growth} = \left[\frac{\text{OD}_{600} \text{ of wells containing drug}}{\text{OD}_{600} \text{ of drug-free well}} \times 100 \right] \dots \dots \dots (1)$$

after the subtraction of ODs of microorganism-free well.

Modulation of antimicrobial resistance was evaluated using the same method, except that the medium was supplemented with sub-inhibitory concentrations (200 µg/mL) of the extracts for the determination of the MICs of ciprofloxacin and ketoconazole. Reserpine at a concentration of 10 µg/mL served as positive control.

The extract modulation factors (MF) were used to evaluate the antimicrobial effects of the extracts on the MIC of the antibiotic.

$$\text{MF} = \left(\frac{\text{MIC antibiotic}}{\text{MIC antibiotic} + \text{modulator}} \right) \dots \dots \dots (2)$$

A modulation factor >2 was set as the cut-off for biologically significant modulation Gröb-

cher *et al.*, 2012).

The fractional inhibitory concentration index (FICI) was used to evaluate the effect of the combination of antibacterial agents

$$\text{FICI} = \text{FIC (standard drug)} + \text{FIC (extract)} \dots \dots \dots (3)$$

$$\text{FIC (standard drug)} = \frac{\text{MIC (standard drug in the presence of extract)}}{\text{MIC (standard drug only)}} \dots \dots \dots (4)$$

$$\text{FIC (extract)} = \frac{\text{MIC (extract in the presence of standard drug)}}{\text{MIC (extract only)}} \dots \dots \dots (5)$$

(Synergy: FICI ≤ 0.5, additive: FICI = 0.5 – 1.0, indifference: FICI = 1 – 2, antagonism: FICI ≥ 2) (Gröblicher *et al.*, 2012).

RESULTS AND DISCUSSION

The 70% ethanol extracts of the three medicinal plants studied showed weak antimicrobial activities against the tested microorganisms (Table 1). The least MIC of 0.28 mg/mL was shown by *C. nudiflora* extract against *E. coli*. The highest MIC of 8.37 mg/mL was registered by *L. schimperi* extract against the same microorganism. Of the three medicinal plants involved in this study, the extract of *C. nudiflora* was the best with MIC values ranging between 0.28 and 1.39 mg/mL. The antimicrobial effects reported here are consistent with similar studies conducted by other workers (Awe and Omojasola, 2009; Babajide *et al.*, 2008; Kisangau *et al.*, 2009; Kuppusamy *et al.*, 2015; Zerbo *et al.*, 2013). Furthermore, the three medicinal plants were investigated for their modulation effects.

Fifteen of the 27 combinatorial cases yielded biologically significant modulation factors causing more than twofold reduction of the MIC of the standard drugs, ciprofloxacin and ketoconazole (Table 2). The highest modulation factor of 70 fold reduction in the MIC of ciprofloxacin was by *C. nudiflora* against *P. aeruginosa*.

Table 1: Mean inhibitory concentration (mg/mL) of different extracts on various microorganisms, n = 3

Test Organism	MIC of antimicrobial agent (mg/mL)		
	<i>L. schimperi</i>	<i>C. nudiiflora</i>	<i>P. reticulatum</i>
Gram positive			
<i>Streptococcus pyogenes</i>	2.29	1.163	1.0
<i>Staphylococcus aureus</i>	2.87	1.353	1.4
<i>Bacillus subtilis</i>	2.28	1.158	0.7
<i>Enterococcus faecalis</i>	2.28	1.182	0.8
Gram negative			
<i>Klebsiella pneumoniae</i>	2.30	1.158	1.6
<i>Escherichia coli</i>	8.37	0.282	3.0
<i>Pseudomonas aeruginosa</i>	2.33	1.393	2.2
<i>Salmonella typhi</i>	3.60	1.259	1.9
Fungus			
<i>Candida albicans</i>	2.99	1.202	1.2

Table 2: MIC values ($\mu\text{g/mL}$) of standard drug plus sub-inhibitory concentration of plant extracts (MF; FICI) for tested microorganisms

Antimicrobial agent	Test organisms								
	Gram positive		Gram negative		Fungus		Fungus		
	<i>S. pyogenes</i>	<i>S. aureus</i>	<i>B. subtilis</i>	<i>E. faecalis</i>	<i>K. pneumoniae</i>	<i>E. coli</i>	<i>P. aeruginosa</i>	<i>S. typhi</i>	<i>C. albicans</i>
Ciprofloxacin	1.39	1.49	1.36	1.39	1.32	0.19	1.40	1.42	
+ <i>L. schimperi</i>	0.55 (2.5; 0.64)	0.86 (1.7; 0.88)	0.30 (4.5; 0.35)	0.32 (4.3; 0.37)	2.73 (0.5; 3.26)	0.19 (0.6; 1.61)	0.30 (4.7; 0.35)	0.31 (4.6; 0.36)	
+ <i>C. nudiiflora</i>	0.05 (27.8; 0.08)	0.075 (19.9; 0.11)	0.07 (19.4; 0.16)	0.78 (1.8; 0.12)	1.70 (0.8; 1.29)	0.07 (2.7; 0.37)	0.02 (70.0; 0.04)	0.55 (2.6; 0.39)	
+ <i>P. reticulatum</i>	0.1 (13.9; 0.2)	2.2 (0.7; 3.2)	1.2 (1.2; 3.2)	2.9 (0.5; 7.3)	2.6 (0.5; 3.2)	0.85 (0.2; 4.46)	1.0 (1.4; 1.8)	0.2 (7.1; 1.2)	
Ketoconazole									1.24
+ <i>L. schimperi</i>									1.24 (1.0; 1.54)
+ <i>C. nudiiflora</i>									0.5 (2.5; 0.7)
+ <i>P. reticulatum</i>									0.5 (2.5; 0.7)

In the modulation assays against the antibiotic susceptible strains, synergistic and additive interactions were found in 13 and 3 of the 27 combinatorial cases respectively (Table 2). These were mainly seen with the extracts of *C. nudiflora* and *L. schimperi*. Five combinatorial cases were indifferent and six were antagonistic. The extract of *P. reticulatum* showed antagonistic and indifferent effects when it was combined with ciprofloxacin against the bacteria strains. However, in combination with ketoconazole, it showed additive effect against *C. albicans*.

The results reported in the present study reveal that the selected medicinal plants bear weak antimicrobial effects. They however demonstrate immense modulation effects in combination with standard antibiotics. These effects observed in the plant extracts may be due to any one or more of the secondary metabolites contained in them (Table 3). Although their exact mechanism of action is not known, we postulate that the synergistic and additive effects observed may be due to the initial damage of the microbial cell wall lipid layer by these secondary metabolites (Trombetta *et al.*, 2005).

This subsequently increases the permeability of the membrane of the microbes and subsequent leakage of intracellular materials. Furthermore, it may increase the accumulation of the antibiotics into the cell and interacting with sites that

are critical for antibacterial effects.

CONCLUSIONS

Altogether the present study revealed that the 70% ethanol extracts of *L. schimperi*, *C. nudiflora* and *P. reticulatum* have weak antimicrobial effects. However, they are good modulators, as they reduced the MIC of ciprofloxacin and ketoconazole by factors that are comparable to those of reserpine, a known modulator. These findings have not been previously reported and these medicinal plants represent potential sources of antibacterial resistance modifying agents. However, the exact compounds and their mechanisms of modulation require further investigation.

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Table 3: Phytochemical constituents present in the plant extracts

Secondary metabolites	<i>L. schimperi</i>	<i>C. nudiflora</i>	<i>P. reticulatum</i>
Phenolics	-	+	+
Saponins	+	+	+
Steroids	+	+	-
Reducing sugars	+	+	+
Flavonoids	+	+	+
Alkaloids	+	-	+
Triterpenes	+	+	+

Key: + means indicated compounds are present, - means indicated compounds are absent

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