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The Effects of the Acidic Metabolites from the Ethanol Root Extracts Lonchocarpus Cyanecsens, Curciligo Pilosa, Securidaca Longipendiculata, Theobroma Cacao on Pseudomonas Aeruginosa

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***Abstract*: The effects of the acidic metabolites of the ethanol extracts of the roots of *lonchocarpus cyanescens, curciligo pilosa, securidaca longipendunculata* and *Theobroma cacao* on *Pseudomonas aeruginosa* was the focus of this investigation. The acidic metabolites obtained by partitioning the crude samples of the root extracts showed that the root of *lonchocarpus cyanescens* contained flavonoids, alkaloids, tannins, saponins, glycosides and phenols. The root of *curciligo pilosa* had saponins, flavonoids, alkaloids, glycosides and phenols. The acidic root extract of *securidaca longipendunculata* indicated the presence of tannins, flavonoids, glycosides and alkaloids. While tannins, saponins, flavonoids, alkaloids, glycosides and phenols were found in *Theobroma cacao*. The antibacterial screening of the extracts showed minimum inhibitory concentration (MIC) at a concentration of 100mg/mL with inhibition zone diameter (IZD) of 36 millimeter (mm) for *lonchocarpus cyanescens*, 00 mm for *curciligo pilosa*, 20 mm for *securidaca longipendunculata* and 30mm for *Theobroma cacao*. The IZD of the reference drug ampiclox at the same concentration of 100mg/mL was 25mm for *lonchocarpus cyanescens* 26mm for *curciligo pilosa*, 20mm for *securidaca longipendunculata* and 28mm for *Theobroma cacao*. *Lonchocarpus cyanescens* gave the highest activity at IZD of 36 mm while *Curculigo pilosa* gave no inhibitory activity.**

***Key words*: Acidic, Antibacterial, Extract, Metabolites, Roots**

1 Introduction

Treatment of infectious diseases becomes more challenging with each passing year. This is especially true for infections caused by the opportunistic pathogen *Pseudomonas aeruginosa*, with its ability to rapidly develop resistance to multiple classes of antibiotics [1]-[4]. Although the import of resistance mechanisms on mobile genetic elements is always a concern, the most difficult challenge we face with *P. aeruginosa* is its ability to rapidly develop resistance during the course of treating an infection.

Infectious diseases have been an important cause of morbidity and mortality throughout our history. With the expansion of the antibiotic era during the 20th century, there was a growing confidence that the need for infectious disease specialists would all but disappear. However, no one could have predicted the impact that an increasing immuno compromised population would have on the resurgence of infectious diseases during the last 3 decades. Furthermore, the ability of bacterial pathogens to adapt and to overcome the challenges of antibiotics in their environment has been nothing short of impressive. We are now faced with a growing population of pan-resistant bacteria that threaten to move us into what some consider the "postantibiotic era" of infectious diseases. Some of the more problematic drug-resistant pathogens encountered today include methicillin-resistant *Staphylococcus aureus*, multidrug-resistant *Streptococcus pneumoniae*, and vancomycin-resistant *Enterococcus* spp. among the gram-positive bacteria and multidrug-resistant *Acinetobacter baumannii*, *Klebsiella pneumoniae*, *Escherichia coli*, and *Pseudomonas aeruginosa* among the gram-negative bacteria.

This continuous evolution of bacteria resistant to currently available antibiotics has become the driving force in the search for more effective compounds that are bactericidal and plants have become the focus. The history of using plants in the treatment of various ailments cannot be over emphasized, since many people rely on them to get relief. All over the world, many people use plant and plant extracts for their antibacterial, antifungal, antiviral, antihypertensive etc activities [5]-[8].

2 Materials and Methods

All reagents were of analytical grade obtained from BDH, England. Weighing was done on Mettler 790 and P1210, powdered sample was obtained by grinding machine model EC-101.

2.1 Plant Sample

Fresh samples 1 kg each of *lonchocarpus cyanescens*, *curciligo pilosa*, *securidaca longipendunculata* and *Theobroma cacao* on *Pseudomonas aeruginosa* were purchased from different localities in Nigeria in November 2013 and authenticated by Prof. C. Onyekwelu of Applied Biology Department Ebonyi State University.

2.2 Extraction

The roots were washed to remove dirt and sand, cut into small pieces, sundried for three days; about 600g of each sample was soaked separately in 2000 mL of ethanol for seven days. After seven days the solution of the extracts was removed by filtration and the solvent distilled off by mild heating to reveal gel like residues: 60 g of *lonchocarpus cyanescens* extract (LCE), 33 g of *curciligo pilosa* extract (CPE), 35 g of *securidaca longipendunculata* (SLE) and 40 g of *Theobroma cacao* (TCE).

2.3 Phytochemical Screening

The presence of the plant chemicals was determined by running classical tests according to the methods [9]-[12].

2.4 Preparation of Acidic Metabolite of the Extracts of LCE, CPE, SLE and TCE

10 g of each plant sample was redissolved in 50 mL of ethanol and introduced into a 250 mL separatory funnel to which were added 20mL concentrated HCl and 30 mL chloroform. The $CHCl_3$ layer was treated with 30 mL 10% NaOH solution; this was shaken and allowed to stand for 3 h. The lower layer was removed and allowed to evaporate to reveal different weights of brownish gel in each case which were redissolved in 30 mL of 96% ethanol, filtered and used without further purification for phytochemical and antibacterial analyses.

2.5 Antimicrobial Test

The antimicrobial test was carried out using the procedure recommended by Holt et al, 1994 and Agah et al, 2011.

3 RESULTS

The phytochemical screening test result is shown on Table I; the inhibition zone diameter (IZD) of the plant species against *Pseudomonas aeruginosa* is depicted on Table II. The IZD values of the control drug at 100mg/ mL are compared with that of the plant species against *Pseudomonas areuginosa* on Table III.

Table I Phytochemicals present in the Acidic Metabolites of the different plant species

Phytochemicals	**Plant Species**			
	L. Cyanescens	*C. pilosa*	*T. cacao*	*S. longependunculata*
Alkaloid 2 mLWagner's reagent +2mL extract	++	+	+	++
Alkaloid 2 mLWagner's reagent +2mL extract	++	+	+	++
Saponin 2 mL extract + 5mL H_2O shaken	+	+	-	+
Glycoside Lieberman test 0.5mL extract + 2 mL $CHCl_3$ + 5mL H_2SO_4	+	+	+	+
Flavonoid 1 mL extract + 5mL dil. NaOH + 3 drops HCl	++	+	+	+
Phenol $FeCl_3$ test	++	+	-	++
Tannin 2 mL extract + 5mL H_2O + 2 drops $FeCl_3$	++	-	+	++

+ = indicated, - = Not indicated.

Table II IZD values of the plant species and Ampiclox against *Pseudomonas aeruginosa*

Plant Species **Concentration 100mg/mL**	***Pseudomonas aeruginosa*** **IZD (mm)**
lonchocarpus cyanecsens,	36
curciligo pilosa	00
securidaca longipendiculata	20
Theobroma cacao	30

00= No activity, 20-36 = Strong activity

Table III IZD values of the plant species and ampiclox against Pseudomonas aeruginosa

Concentration 100mg/mL	***Pseudomonas aeruginosa*** **IZD (mm)**
lonchocarpus cyanecsens, Ampiclox	36 25
curciligo pilosa Ampiclox	00 26
securidaca longipendiculata	20

Ampiclox	20
Theobroma cacao	30
Ampiclox	28

00= No activity, 20-36 = Strong activity

4 DISCUSSION

The phytochemical screening of the ethanol root extracts of the plant samples indicated the presence of alkaloid, saponin, glycoside, flavonoid, phenol and tannin. CPE did not indicate the presence of tannin while saponin and phenol were not present in SLE. Alkaloid, tannin and flavonoids have been discovered to exhibit a wide array of physiological activities such as antibacterial activity, antifungal potency, wound healing property, in the treatment of sexually transmitted diseases, and cure for skin diseases. The antibacterial inactivity of the acidic metabolite of CPE might be attributed to the absence of tannin [13]-[15]. The crude sample of CPE has shown antibacterial activity against *Escherichia coli, Staphylococcus aureus, Streptococcus faecalis and Pseudomonas aeruginosa* [16].

The antibacterial activity of the plant samples showed that LCE had the greatest inhibitory activity at IZD 36 mm; the extract had alkaloid, flavonoid phenol and tannin as phytochemicals which working in synergy showed a high potency against this opportunistic microorganism. TCE had IZD 30 mm which was also bactericidal against *Pseudomonas aeruginosa;* a plant sample becomes bacteriastatic at IZD values between 15-18 mm. SLE was also active but not as lethal against the microorganism as LCE. CPE showed zero activity against *Pseudomonas aeruginosa*.

Comparing the IZD values of the plant samples with the reference drug ampiclox at the concentration of 100 mg/mL it was observed that LCE and TCE were more active against the standard while SLE showed equal potency. The choice of this particular antibiotic is because it is the commonest drug recommended by doctors in the treatment of gram-negative and gram positive bacteria.

The active metabolites from these plant samples might be looked at as potential antibacterial drugs of the future to fight against *Pseudomonas aeruginosa* which is posing a threat because of its resistance to some known antibiotics.

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