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Antibacterial (spectrum) activity of some medicinal plants against extended spectrum beta-lactamase producing *Escherichia coli, Klebsiella pneumonia* and *Proteus mirabilis*

Bassey Ini Ubi and Andy Iniobong Ebenge

Department of Microbiology, University of Calabar, Calabar Cross River State, Nigeria

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ABSTRACT

This study is aimed at accessing the antibacterial activity of some medicinal plants against extended spectrum beta-lactamase producing enterobacteriaceae that are prevalent in Calabar hospitals. Aqueous leaf extracts ofEditan (Lasiantheraafricana),Ikongetidot (Veroniaamygdalina) and Atama (Heinsiacrinata) were used for the ESBL testing. Ikongetidot (Veroniaamygdalina) showed significantly highest antibacterial activity against extended spectrum beta-lactamase producing E. coli and K. pneumonia (17mm-16mm) respectively, but also significant against proteus. While Editan (Lasiantheraafricana) and Atama (Heinsiacrinata) showed least antimicrobial activity against all the test organisms. Aqueous extracts of Ikongetidot produces largest zones of clearance on E.coli, K. pneumonia, and Proteus mirabilisfollowed by Editan and atama, prodicng a statistically different ($p<0.5$) values. These findings present supports for the possible use of the aqueous extract of IkongEtidot, Editan and Atama for the detection of ESBL producers from clinical isolates.

Keywords: Medicinal plant, aqueous extract, ESBL, antimicrobial activity.

INTRODUCTION

In developing countries of Africa, a number of people still rely on traditional herbalists for their medical care. In Cross River State, traditional medicine has played a pivotal role in preventing and eliminating various kinds of diseases especially in rural areas. Plants contain chemical constituents that have great potential for medicinal use and both traditional healers and pharmaceutical drug companies explore these plants widely [1].

The increasing resistance of bacterial isolates to antibiotics including the broad spectrum and extended broad spectrum antibiotics is a quest for a search on an alternative antimicrobial agents that can inhibit or kill these resistant strains of bacteria, especially ESBL (extended spectrum beta-lactamase producers).

Medicinal plants have been found to possess some therapeutic agents including tannins, alkaloids, Saponinscardiac glycosides, Anthraquinones, Terponoids, flavonoids and reducing sugar. [2].In a separate study by[3], *Garcinia kola* (roots), *Borreriaocymoides* (leaves), *Kola nitida* (bark) and *Citrus aurantifolia* (roots) screened for phytochemical components were found to contain tannins, phlobatannins, polyphenols, hydroxymethylanthraquinones, glucides, saponins, alkaloids, cardiac glycosides, flavanoids and reducing compounds. These bioactive agents are said to inhibit the growth of microorganisms including extended spectrum beta-lactamase producing organisms since they are possess both antimicrobial and antifungal activity [4]. The main purpose of the present study is aimed at accessing the antimicrobial activity of three medicinal plants against beta-lactamase producing enterobacteriaceae that are prevalent in Calabar hospitals.

MATERIALS AND METHODS

Sample collection (collection of ESBL samples)

Bacterial samples of *Escherichia coli*, *Klebsiella pneumonia* and *proteus mirabilis* were collected from in-patients and out-patients of General Hospital Calabar, Teaching Hospital Calabar and University of Calabar Teaching Hospital. Gram staining technique biochemical characterization was used to identify the organisms.

Collection of Plants

Ikongetidot (*Veroniaamygdalina)*,Editan (*Lasiantheraafricana)* and atama (*Heinsiacrinata)* were collected from UNICAL farm and identified in botany department, University of Calabar.

Preparation of extracts

The harvested leaf were properly washed with clean water and then dried under the sun. It was then blended into powdered form used for extraction with aqueous solvent and methanolic solvent using soxhlet apparatus extractor. The solvent was then water bath at 70-80^{o}C to get a semi-solid crude extract.

Extended spectrum beta-lactamase detection

Agar well diffusion was employed for the detection of ESBL using the crude plant extracts. From the crude extract, 500mg/ml dilution of the plant paste was prepared for the detection. 200µl (500mg/l) of the extract were poured into the well and incubated at 37^{o}C for 24hrs. The plates were then observed after 24hrs incubation for zone of clearance. The zones were measured and recorded in millimeter.

RESULTS

Table 1:Distribution of organisms in GHC THC and UCMC

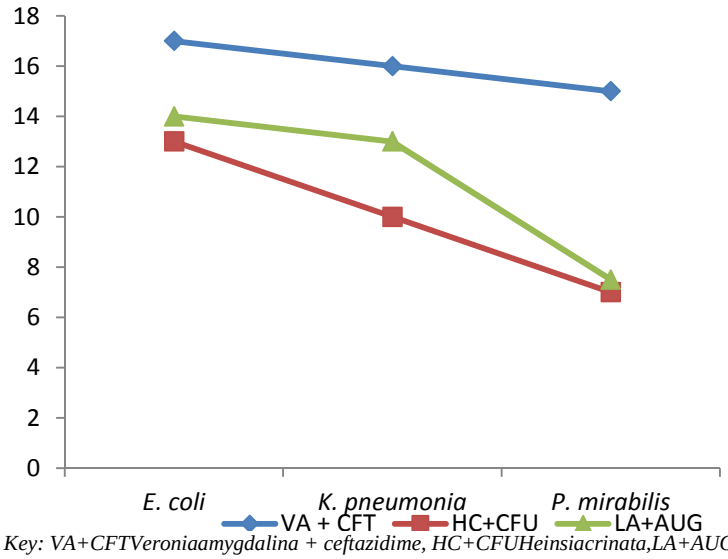
Organisms	GHC (%) n=89	THC (%) n=75	UCMC (%) n=73	Total (%) n=237
E. coli	49(55.05)	42(56.05	47(64.38)	138(58.22)
K. pneumonia	26(29.05)	22(29.33)	18(24.65)	66(27.84)
Proteus mirabilis	14(15.73)	11(14.66)	8(10.95)	33(13.92)

Key: *GHC - General Hospital Calabar, THC – Teaching Hospital Calabar, UCMC – University of Calabar Medical Centre.*

Table 2: Positive ESBL Isolates from In-patients and out-patients

Organisms	GHC (%)	THC (%)	UCMC (%)	Total (%) n=143
E. coli	32	26	21	79(55.24)
K. pneumonia	21	16	11	48(33.56)
Proteus mirabilis	8	5	3	16(11.18)

Key: *GHC - General Hospital Calabar, THC – Teaching Hospital Calabar, UCMC – University of Calabar Medical Centre.*



Key: VA+CFTVeroniaamygdalina + ceftazidime, HC+CFUHeinsiacrinata,LA+AUGLasiantheraafricana

Fig.1: Graph showing different antimicrobial activity of the plant extract in combination with Ceftazidime, Cefuroxime and Augmentin.

Table 3: Antibacterial activity of medicinal plant

Organisms	Ikongetidot (*Veroniaamygdalina)* Zone of inhibition (mm)	Atama (*Heinsiacrinata)* Zone of inhibition (mm)	Editan (*Lasiantheraafricana)* Zone of inhibition (mm)	Total (%) n=104
E. coli	17	13	14	42(40.38)
K. pneumonia	16	10	13	36(34.61)
Proteus mirabilis	15	9	7.5	26(25.00)

Table 4:Confirmation of organism based on biochemical tests

Organisms	Gram strain	Citrate test	Indole test	Oxidase test	Catalase test
E. coli	-	-	+	-	+
K. pneumonia	-	-	-	-	+
Proteus mirabilis	-	-	-	-	+

DISCUSSION

The emergence of ESBL-producing coliforms since the mid-1980s till date has over 100 types of different enzymes described and have become a world wide clinical issue to contend with. However, the members of the enterobacteriaceae producing ESBL are a clinical threat and have been associated with increasing mortality in both in patients and out patients with severe infections [5].

Distribution frequencies of ESBL producing isolates from the sampled hospitals in Calabar employed for the antimicrobial testing of the three selected medicinal plants (Editan, Ikongetidot, and atama) revealed that *Escherichia coli* had the highest with distribution frequency of 138(58.22)% followed by *Klebsiella pneumonia* 66(27.84)% with *Proteusmirabilis*having the least distribution frequency of 33(13.92)% (Table 1.0). Considering the distribution of isolates amongst the sampled hospitals in Calabar, from table 1, General Hospital Calabar had the highest distribution frequency of 49(55.05)% for *E. coli,* 26(29.21)% for *K. pneumonia* and 14(15.73)% followed by Teaching Hospital with distribution frequency of 42 (56.00)% for *E. coli,* 22(29.33)% for *Klebsiella pneumonia* and 11(14.66)% for *Proteus mirabilis.* However, University of Calabar teaching hospital Calabar had the least distribution frequency of 47(64.38)% for *E. coli,* 18(24.65) for *Klebsiella pneumonia* and 8(10.95)% for *proteus mirabilis* respectively.

From table 2, the results of the ESBL confirmation test using Agar diffusion with broad spectrum Cephalosporins, (ceftazidime, cefotaxime and cefuroxime) and Augmentin showed that *Escherichia coli* had the highest positive extended spectrumbeta-lactamase producers of 79(55.24)% followed by *Klebsiella pneumonia* with 48(33.56)% and 16(11.18)% for *Proteusmirabilis* respectively for the three hospitals.

Results obtained from this study vividly showed that the three plants extract used for the study had a significant antimicrobial activity against the test ESBL bacteria (*E. coli, K. pneumoniaand P. mirabilis)* (Table 3).From table 3, the maximum zone of inhibition was observed in the methanolic extract of (*Veroniaamygdalina* (Ikongetidot) against *Escherichia coli* (17mm), *Klebsiella pneumonia* 16mm and *proteusmirabilis* 15mm respectively. A similar study carried out by [6] using aqueous extracts of *Terminaliachebula*and Z*inziberofficinale* indicates maximum zones of inhibition against *proteusmirabilis* as 1mm, 9mm and 0mm for *E. coli,* 5mm and 5mm for *Pseudomonas aeruginosa*and 5mm and 6mm for *Klebsiella pneumonia,* respectively. Hence, the studied plant extracts had shown more effective results against ESBL producing strains of bacteria and thus can be used pharmaceutically to produce drugs for ESBL related diseases. In another study by [7] using methanolic extracts of Thevetiaperuviana showed maximum zone of inhibition against *Klebsiella*(15mm), 14mm for *E. coli, Proteusmirabilis*and MRSA and 13mm for *Pseudomonas*respectively. In another study done by [8] using aqueous extract of *Veroniablumeoides* and *Phyllanthusannarus* against methicilin resistant *staphylococcus aureus*(MRSA),11mm and 13mmwas observed respectively as maximum zones of inhibition. This study again shows a less potency of the plant extract than the studied plant extracts.

The emergence of ESBL producing pathogenic enterobacteria species in Calabar Hospital/Clinics had posed a serious antibiotic management, hence creating treatment failures by the use of even third generation Cephalosporins (3GCs). This is a wake-up call for laboratory testing and detection methods that will accurately identify the presence of these oxyimino enzymes in clinical isolates of Calabar Hospitals. Our study on the three medicinal plants had proven that the extracts can be used for both ESBL detection, confirmation and pharmaceutical production of drugs to treat diseases/infections associated with ESBL. The potency of medicinal plants against ESBLs producing bacteria was again demonstrated by [9] using little amounts of flavonoids separated by TLC which weresufficient to produce inhibitory effects on the growth of ESBLs producing bacteria. Although few workers have reported on the synergistic interactions of plant extracts/phytocompounds with antibiotics from different part of the world [10]; [11];

[12], *VeroniaAmygdalina, Heinsiacrinata,Lasiantheraafricana* indicate a promising interaction in combination with Ceftazidime (VA+CFT), Cefuroxime (HC+CFU) and Augmentin (LA+AUG) with high antimicrobial activity on the test organisms (*E. coli, K. pneumonia* and *P. mirabilis*) as shown in Fig1. The effectiveness of our three studied medicinal plants extracts against ESBL producing bacteria can also be attributed to the high percentage of flavonoids and alkaloids.

CONCLUSION

The medicinal plants used for this study has demonstrated a very high efficacy against ESBL producing bacteria. Their exploration would be very much useful to a greater percentage of both rural and urban dwellers that solely rely on traditional medicine as their first line therapy. Above all, further studies need to be done on a better way to preserve the extract from deterioration in case of further use.

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