ACTIVITIES OF EXTRACTS OF NEGRO-PEPPER (*XYLOPIA AETHOPICA)* AND CASHEW (*ANARCARDIUM OCCIDENTALE*) ON SOME ANTIBIOTIC RESISITANT CLINICAL ISOLATES FROM BAPTIST MEDICAL CENTRE, EKU, DELTA STATE, NIGERIA

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Abstract
Aqueous and ethanolic extracts of *Xylopia aethiopica* an *Anacarduim occidentale* obtained from Abraka main market, Delta State were analysed for their activities against *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*. The phytochemical analysis of the plants showed that they contain Alkaloids, Tannins, Saponins, flavonoids, Glycosides and chalcones. The antibiotics sensitivity pattern of *S.aureus* revealed resistance against Augmentin, Amoxycillin, Cloxacillin, Cotrimoxazole while *E.coli*, *K.pneumoniae* and *P.aeruginosa* were all resistant to Nalixidic and Ampicillin and had varying degrees of resistance to other antibiotics. *S.aureus* was sensitive to all the extracts at a minimum concentration of 12.5 % (v/v) of A. occidentale and at 50% (v/v) of x.aethiopica at minimum inhibitory concentration of 25% (v/v). *E. coli* was resistant to all the extracts but growth of all the organisms was inhibited when the extracts were applied in combination.

INTRODUCTON

Research and Development is finding it difficult to produce new drugs to treat bacteria that become resistant to current antibiotics. Infectious diseases physicians are alarmed by the prospect that effective antibiotics may not be able to treat seriously ill-patients in the near future. The resistance problem demands that a renewed effort be made to seek antibacterial agents effective against pathogenic bacteria resistant to current antibiotics (Poole, 2004). One of the possible strategies is the use of bio-active phytochemicals which are available in plants.(Singh *et al.,* 2009). The medicinal value of plants lies in some chemical substances that produce a definite physiological action on the human body. The most important of these bioactive compounds of plants are alkaloids, flavanoids, tannins and phenolic compounds. Traditional medicine claims to have used plants in the treatment of diseases such as stomach pain, dysentery, urinary tract infection, skin diseases, venereal diseases, influenza, diarrhea, respiratory infections and a host of others.

This research reports the methods and results of the use of *X.aethiopica* and *A. occidentale* against some clinical isolates.

MATERIALS AND METHODS

Collection of Samples

Negro Pepper (*Xylopia aethiopica)* fruits were got from Abraka main market, Delta State while the Cashew leaves were plucked from the senior staff quarters, Delta State University, Abraka. They were identified at the Botany Department, Delta State University, Abraka. The clinical isolates were obtained from the Microbiology laboratory of Baptist Medical Centre, Eku, Delta State. They were transported to the laboratory in ice packs and stored in the refrigerator at 4^0C.

Plant Extraction

This was done using a modified method of Aboaba *et al*. (2006).The plant materials were air-dried on a disinfected surface for four days and ground to powder. – 100g of the powder was mixed with each of 50 ml of 95% ethanol and distilled water for ethanol and water ex-

tract respectively. They were shaken on an orbital shaker for 3 days. They were filtered using Whatman No. 1 filter papers. The filtrates were concentrated over a steam-bath to form sticky semi-solid paste. This was stored in the refrigerator at 4^0C. The extracts were labeled CWE (Cashew Aqueous Extract), CEE (Cashew Ethanolic Extract), NEE (Negro Pepper Ethanolic Extract) and NEW (Negro Pepper Aqueous Extract)

Stock Culture Preparation

The clinical isolates were inoculated on sterilized Nutrient Agar in McCartney bottles and incubated for 24 hours at 37°C. They were subsequently kept in the refrigerator at 4^0C.

Sensitivity Test

The disc diffusion method was used (Fawole and 0so, 2007). Sterilized forceps was used to place the disc in different concentrations (12.5%, 25% and 50% v/v) of the extracts. The discs were then aseptically placed on sterilized Nutrient Agar plates. Each plate was seeded with each test organism and incubated at 37^0C for 24 hours. Zones of inhibition were measured. The minimum inhibitory concentration of each extract on the clinical isolates was also determined.

Phytochemical Analysis of Extracts

The extracts were tested for Alkaloids, Tannins, Saponin, Flavonoids, Glycosides and Chalcones as described by Trease and Evans (2002) and Fasoyinbo and Adegoke (2007).

Effect of combination of extracts on isolates.

Combinations of the extracts were prepared as follows:

0.2 ml of CWE with 0.2ml of NEW
0.2ml of CWE with 0.2ml of NEE
0.2ml of NEW with 0.2ml of NEE
0.2ml each of the extracts mixed together.

Each combination was shaken vigorously and paper discs were impregnated and allowed to dry. The dried disks were carefully placed on Sterile Nutrient Agar plates seeded with the test organisms. The plates were incubated at 37^0C for 24 hours.

Antibiotic sensitivity of the isolates testing

Gram positive and Gram negative antibiotic discs from Abtek Biological Limited (Nigeria) were used. The discs were carefully placed on Nutrient Agar plates seeded with the test organisms and incubated at 37°C for 24 hours.

RESULTS AND DISCUSSION

The phytochemical analysis of the extracts (Table 1) showed that all the extracts contain Alkaloids, Tannins and Glycosides. NEE does not contain saponin, while NEW and CEE do not contain flavonoids. Only CEE does not contain chalcones. This conforms with the findings of Ijeh *et al*. (2004) that *X.aethiopica* is rich in alkaloids, tannins, flavonoides, steroids, oligosaccharides and cyanogenic glycosides. Cashew fruit possesses volatile compounds which include esters, terpenes and carboxylic acid (Bicalho, 2001) while the back and leaves are rich in tannins ,anacardic acids are found in the nutshell. Cashew also contains amino acids, anacardol, antimony caprylic acid, garlic, hexanal, linoleic acid, stearic acid (Bicalho, 2001)

Table 1: Phytochemical Analysis of the Plant Extracts

	Extracts			
Phytochemical Compound	NEW	NEE	CWE	CEE
Alkaloids	+	+	+	+
Tannins	+	+	+	+
Saponins	+	-	+	+
Flavonoids	-	+	+	-
Glycosides	+	+	+	+
Chalcones	+	+	+	-

Key:

+	-	**Present**
_	-	**Absent**

Table 2: Zones of Inhibition (mm) of the plant extracts on the test organisms Diameter of Zones of Inhibition (mm) on test organisms

Extract	S.aureus	E. coli	K.pneumonia	P.aeruginosa
NWE	10	-	9	-
NEE	9	-	8	9
CWE	18	-	8	-
CEE	15	-	-	-

Key:

- ---------- No Inhibition

Table 2 shows the inhibitory effect of the extracts on the test organisms.

S. aureus was susceptible to the four extracts

with the zones of inhibition ranging from nine millimeters for NEE to eighteen millimeters for CWE. *P. aeruginosa* was sensitive to only one of the extracts (NEE). *E. coli* showed resistance to all the extracts.

The phytochemical compounds present in these plant substances have been shown to have high inhibitory effects on the growth of micro-organisms (Edeoga and Eriata, 2001). Tannins exhibit high oxidative inhibitory activity against micro-organisms due to the presence of gallic and digallic acids (Ijeh *et al.,* 2004). Resistance of *E.coli* to all the extracts might be attributed to the fact that some *E. coli* serotypes through the use of efflux pumps and other mechanisms are not affected by many anti-microbial agents.

Table 3: Effects of different combinations of Extracts on the test organisms Organisms

EXTRACT	*S.aureus*	*E. coli*	*K.pneumoniae*	*P.aeruginosa*
NEW/CWE	+		+	+
NEE/NEW	+		+	+
CWE/NEE	+		+	+
CEE/CWE/NEE/NWE	+	+	+	+

Key:- + - Sensitive

All the organisms were inhibited by the different combinations of the extract . The sensitivity might be attributed to an increase in the concentration of the antimicrobial compounds and the combined effect might have conferred a broad – spectrum activity on the extracts.

Table 4:. Minimum Inhibitory Concentration of the extracts on the test organisms

Extracts

	CEE			CWE			NEE			NEW		
Organisms	12.5%	25%	50%	12.5%	25%	50%	12.5%	25%	50%	12.5%	25%	50%
S.aureus	6	10	12	6	9.0	12.0	-	-	8.0	-	-	9.0
K.pneumoniae	-	-	-	-	-	6.5	-	-	6.0	-	6.0	8.0
P.*aeruginosa*	-	-	-	-	-	-	-	6.0	7.5	-	-	-
E.coli	-	-	-	-	-	-	-	-	-	-	-	-

Table 5: Antibiotic Sensitivity test on Gram negative test organism

Antibiotics	**E.coli**	**K. pneumoniea**	**P.aerugims**
Ciproflaxin (10mg)	+	-	+
Streptomycin (10mg)	-	+	_
Septrin 30mg)	+	_	+
Ampicillin (30 mg)	-	_	_
Tarivid (10mg)	+	+	+
Ceporix (10mg)	+	_	+
Gentamycin (10 mg)	+	+	_
Augmentin (30mg)	+	_	+
Nalixidic acid (30mg)	_	_	_
Peflacine (10mg)	+	+	+

Key:

+	-	**Sensitive**
_	-	**Resistant**

Table 6 shows the result of the antibiotic sensitivity pattern of *S. aureus* using a gram positive antibiotic disk IVD 1.0 GBMTS – POS. The test revealed that *S. aureus* was resistant to augmentin, Amoxycillin, cloxacillin and clotrimazole but sensitive to tetracycline and chloramphenicol. (Poole, 2004).

Some strains of *S. aureus* developed resistance to antibiotic through production of B-lactamases which disrupt the B-lactam ring structure of B-lactam drugs and through the production of unique penicillin binding protein that has low affinity for the B-lactam antibiotics (Prescott *et al.,* 2005).

Table 6: Antibiotic sensitivity test on S.aureus

Antibiotics	**Effect**
Augmentin (30mg	_
Amoxycillin (25mg)	_
Erythromycin (5mg	+
Tetracycline (10mg)	++
Cloxacillin (5mg)	_
Gentamicin (10mg)	+
Cotrimoxazole (25mg)	_
Chloranephenicol (30mg)	++

Key:

+	-	Sensitive
++	-	Very sensitive
_	-	Resistant

Table 6 shows the antibiotic sensitivity pat-

tern of the Gm positive organisms. There was varying degrees f resistance among all the organisms. *P. aeruginosa* has been found to exhibit low antibiotic susceptibility. This is attributable to a concerted action of multi-drug efflux pumps with chromosomally – encoded antibiotic resistance genes and the low permeability of the bacterial cellular envelopes. 80% of *E.coli* have being shown to be resistant to one or more drugs (Poole, 2004).

Conclusion

The development of resistance of pathogens to many commonly used drugs is amajor global health problem. There is therefore the need for newer drugs or agents that will combat these organisms and pose little or no risk to the patient. Extracts of *X. aethiopica and A. occidentale* of natural origin were found to possess activities *against E.coli, S.aureus, K.Pneumoiae and P.aeruginosa.* Different contributions of the extracts were quite effective in inhibiting the growth of these organisms. These plants are cheap, available so can be formulated into preparations of effective known concentrations that can be used in the treatment of infections caused by these organisms. They are of plant origin, used in the food industry so might be 'body friendly'

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