Piper Guineense: A Possible Alternative Treatment for Multidrug Resistant EHEC

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Abstract

The susceptibility of sixteen (16) Enterohaemorrhagic Escherichia coli 0157: H7 isolates to conventional antibiotics and extracts of P. guineense was compared using agar diffusion method. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the extract was determined using broth dilution and agar diffusion methods respectively. The result of the susceptibility test showed that majority of the EHEC isolates were resistant to 63.64% of the antibiotics used, showing multidrug resistance. The antibacterial assay of P. guineense revealed inhibition against all the isolates (100%) with MIC (200mg/ml) and MBC (400mg/ml).

Key Words: EHEC 0157: H7, Multidrug Resistance, Antibiotics, *Piper guineense*, Diarrhoea.

Introduction

Diarrhoea poses a very serious problem in developing countries where it is among the leading cause of morbidity and mortality among children. According to the federal statistics bulletin of Nigeria, it ranks second as a major cause of morbidity among the notifiable diseases in Nigeria where three hundred (300) children die every day from dehydration and malnutrition resulting from diarrhoea (Emejuaiwe *et al.*, 1981).

Escherichia. coli is one of the bacterial causative agents of diarrhoea. At least five different categories of *E. coli* may cause diarrhoea: enterotoxigenic *E. coli* (ETEC), Enterohaemorrhagic *E. Coli* (EHEC), enteropathogenic *E. coli* (EPEC), enteroinvasive *E. coli* (EIEC), and enteroaggregative *E. coli* (EAEC) (Nataro and Kaper, 1998). Enterohaemorrhagic *E. coli* (EHEC) is one of the five groups of *E. coli* which are recognized as important etiological agents of diarrhoea and its discovery is one of the most important landmarks in the field of enteric infection (Mohammed *et al.*, 1986). Iruka *et al*., 2003 isolated 330 *E. coli* from children with diarrhoea, out of which 2(0.6%) were EHEC and other *E. coli* that hybridized with shiga toxin gene probe but lacked other characteristics of EHEC constituted 28(8.4%) of the total *E. coli* isolated. He demonstrated that EHEC is an important diarrhoea pathogen among adults in south western Nigeria.

Much work has been done on medicinal plants in Nigeria and Africa (Ilori *et al.*, 1996; Adeleye *et al.*,2003; Akinyemi *et al.*, 2005). Plants are used in treating malaria, diarrhoea, burns, gonorrhoea, stomach disorders and other infectious diseases. *Piper guineense* has insecticidal activity against *Zonocerus variegatus* which is attributable to the piperine-amide composed by the plant. The leaves are considered aperitive, carminative and eupeptic. They are also used for the treatment of cough, bronchitis, intestinal diseases and rheumatism (Sumathykutty *et al*., 1999).

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According to World Health Organization (WHO) more than 80% of the world's population relies on traditional medicine for their primary healthcare needs. The use of herbal medicines in Asia and Africa signify a long history of human closeness and interactions with the environment.

The development of drug resistance in human pathogens against commonly used antibiotics has necessitated a search for new antimicrobial substances from other sources including plants. Therefore screening of medicinal plants for antimicrobial activities and phytochemical properties is important for finding potential new compounds for therapeutic use (Adeleye *et al.*, 2003).

Considering its role as causative agent of diarrhoea EHEC has now become very important in Nigeria, hence the need to study the antibiotics susceptibility and resistant pattern of this important organism and find an alternative treatment for the resistant strains.

2.1 Materials and Methods

2.1.1 Sample Collection and Preparation of Plant Extract (*P. guineense)*

Plant was purchased from a local market in Lagos and identified at the Department of Botany, University of Lagos, Nigeria. The plant material was oven dried at 40°C for 2weeks after which it was grinded into powder and soaked in sterile water at 4°C for 1week, sieved with white handkerchief and the residue discarded. The supernatant was further filtered with Whatman filter paper no.1 and freeze dried. The solid extract was weighed and 4.0g was added to 10mls of water to give a concentration of 400mg/ml. This was kept as the stock solution of the extract.

2.1.2. Bacterial strains

All the EHEC isolates used for this research were obtained from Molecular Biology / Biotechnology Division of Nigerian Institute of Medical Research, Yaba, Lagos.

2.1.3 Preparation of bacterial culture

The stock culture of the bacteria used was sub-cultured on sorbitol- MacConkey agar plates and incubated at 37^0C for 24 hours.

2.1.4 Antibiotics SusceptibilityAssay

The test organisms were inoculated into Brain heart infusion broth (Oxoid) and incubated for 18hours at 37°C. The 18hr broth culture was centrifuged (3000rpm) to pellet cells and supernatant was discarded. Cells pellet was washed with phosphate buffered saline three times to remove debris. The washed cells were re-suspended in phosphate buffered saline (PBS) and standardized using McFarland's standard tube No. 0.5 to give an inoculum size of $1x10^8$CFU /ml. Standard inoculums were seeded on Mueller Hinton agar (Oxoid). Antibiotics multidisc (Abtek, Liverpool) was placed on the seeded plates and incubated for 24hours at 37°C.The susceptibility of the test bacteria strains to various antibiotics was performed following National Committee for Clinical Laboratory Standard Recommendation (NCCLS, 2006a).

2.1.5 Antibacterial activity Assay of *Piper guineense* extract

The standard inoculum of the test organism for the plant assay was prepared as described above. The test organism was seeded on Mueller Hinton agar (Oxoid). Wells were borne into the agar with a sterile cork borer (5mm).The wells were labeled according to the extract concentrations. The extracts were dispensed into the holes and the plates were incubated at 37°C for 24 hours. Clear zones surrounding the wells indicated the sensitivity of the organism to the plant extract, while lack of clear zone indicated resistance to the extract.

2.1.6 Determination of Minimum inhibitory concentration (MIC) and Minimum bactericidal concentration (MBC):

The extract was double serially diluted in Brain Heart Infusion broth (Oxoid) and inoculated with 20µl of the standard inocula. After 24hours of incubation, the tubes were observed for visible turbid growth (Ilori *et al.*, 1996).

3.0 Results

All the sixteen organisms exhibited multi drug resistance to five or more of the antibiotics used but none was resistant to the extract of *P. guineense*. The most effective of the eleven antibiotics were Ofloxacin and Colestin Sulphate which were active against 15(93.4%) of the isolates; followed by Nitrofurantoin with 14(87.5 %), Gentamycin 7(56.3%), Ciprofloxacin 6(43.8%), Tetracycline 2(12.5%), Augmentin, Cotrimoxazole, Ampicillin and Streptomycin 1(6.3%). *P. guineense* was active against all the EHEC isolates used. This is shown in Table I. The comparism of the zones of inhibition exhibited by *Piper guineense* and the various antibiotics used on EHEC is shown on Table II.

3.1 Determination of MIC and MBC

The average MIC of the extract was 200mg/ml while the MBC was 400mg/ml.
The number of organisms showing resistance compared with the number of organism sensitive to the different antibiotics are shown on Figure I.

4.0 Discussion

The activity of plant extracts against bacteria has been studied for years but in a more intensified way during the last three decades. During this period numerous antimicrobial screening evaluations have been published based on the traditional use of Chinese, African and Asia plant-based drugs (Suffrendini *et al.,* 2004). A large number of constitutive plant components have been reported to have antimicrobial activity. Well known examples include phenols, unsaturated lactones, saponins, cyanogenic glycosides and glucosinolates (Adejumobi *et al.,* 2008*)*. The inhibitory effect of *P. guineense* extract on some test EHEC strains was investigated in vitro. EHEC are increasingly isolated from severe diarrhoea disease and constitute a serious medical problem for many patients (Paton and Paton, 1998; Boerlin *et al.,* 1999). Infection with these organisms may result in life-threatening complications such as hemolytic-uremic syndrome (HUS) and thrombotic thrombocytopenic puerperal (Paton and Paton, 1998).The results obtained in this study revealed the antibacterial potential of this extract. The antibacterial assay of *P. guineense* revealed potency against all the sixteen strains of EHEC screened at MIC concentration of 200mg/ml and MBC of 400mg/ml.

The antimicrobial properties of substances are desirable tools in the control of infections and in food spoilage (Aboaba *et al.,* 2005).The high level of sensitivity observed in the aqueous extract towards the bacterial pathogens showed that the active components were soluble in water. The influence of solvent for extraction on the inhibitory capacity of the extract on the test organism has been reported by Al-Bayati and Sulaiman (2008).

It is believed that the *P. guineense* stimulates the production of hydrochloric acid in the stomach and promotes the health of the digestive tract. Plant based antimicrobials have enormous therapeutic potentials as they can serve the purpose with lesser side effects that are often associated with synthetic antimicrobials (Iwu *et al.,* 1999). The active components of these extracts usually interfere with the growth and metabolism of microorganisms in a negative manner and are quantified by determining the minimum inhibitory concentration and the minimum bactericidal activity. These values are used as guide for the treatment of most infections (Aboaba *et al.,* 2005).
The result of the antibiotics susceptibility showed that only four (36%) antibiotics were potent against majority of the EHEC while the organisms showed multidrug resistance to the remaining seven (64%) antibiotics. The result of the antibiotics susceptibility revealed that most of the isolates carry multidrug resistant gene which could be due to gross misuse of these antibiotics. This agrees with the work of Smith *et al.,* 2003. There was no significant difference between the zones of inhibition of Ofloxacin and *P. guineense* but there was a very great difference between *P. guineense* and the remaining ten antibiotics when the zones of inhibition were compared.

Comparing the sensitivity of the bacterial strains to both the plant extract and synthetic antibiotics, the result showed that the plant extract can be used as an alternative to the antibiotics as the zones of inhibition shown were greater than those of the antibiotics and the plant extract will have a lesser side effect than those associated with the use of antibiotics (Marchese and Shito, 2001; Poole, 2001). Also the issue of resistance to the plant extract cannot arise as is found with antibiotics (Kareem *et al.,* 2010). The results obtained support the fact that further work needs to be done to determine and identify, purify and quantify the antibacterial compound within *P. guineense* and also to determine its full spectrum of efficacy.

Conclusion

The aqueous extracts of *P. guineense* show promise and form a primary platform for further phytochemical and pharmacological studies for use as alternative therapy for infections caused by EHEC.

Table 1:-Antibiotics Susceptibility Pattern and Antibacterial activity of *P. guineense* in mm

CODE	TET	AUG	AMP	COL	OFL	GEN	NAL	NIT	COT	CIP	STR	PG
H21	0	0	0	13	25	0	0	25	0	0	0	26
H22	0	0	11	14	28	15	20	22	0	25	0	24
H24	16	0	0	14	29	0	0	0	0	19	0	29
H25	0	0	0	13	26	20	22	18	0	0	0	25
H26	0	0	0	12	24	23	18	14	0	20	0	24
H27	14	0	0	11	29	19	0	24	0	26	0	28
H30	0	0	0	14	30	0	0	26	0	24	0	23
H32	0	0	0	12	25	0	0	0	11	20	14	27
H33	0	0	0	12	32	22	0	18	0	0	0	26
H35	0	0	0	11	24	14	0	20	0	0	0	25
H36	0	0	0	10	19	23	23	19	0	25	0	22
H38	0	0	0	12	27	20	0	23	0	0	0	28
H39	0	20	0	14	28	24	0	22	0	0	0	24
H40	0	0	0	14	30	20	22	18	0	0	0	30
H49	0	0	0	11	26	0	0	19	0	0	0	23
H55	0	0	0	0	0	0	21	0	0	0	0	22

KEY

TET…Tetracycline STR…Streptomycin AUG…Augumentin P.G…*Piper guineense* AMP…Ampicillin COL…Colesti OFL…Ofloxacin GEN…Gentamytcin NAL…Nalidixic Acid NIT…Nitroforantoin COT…Cotrimoxazole CIP…Ciprofloxacin

Table 2: Comparison of Mean ±Standard Deviation of zones of inhibitions of the antibiotics with *P. guineense*

DRUGS	MEAN ±SD.	t-VALUE	pVALUE
PIPER GUINEENSE	25.375±2.473		
TET	1.875±5.136	21.089	0
AUG	1.2500±5.00	16.362	0
AMP	0.6875±2.75	24.926	0
COL	11.687±3.381	17.008	0
OFL	25.125±7.384	0.155	0.879
GEN	12.500±10.328	4.947	0
NAL	7.850±10.551	6.099	0
NIT	16.75±8.851	3.664	0.002
COT	0.688±2.750	29.382	0
CIP	9.938±11.784	5.051	0
STR	0.875±3.500	25.027	0

$p<0.05$ is statistically significant.

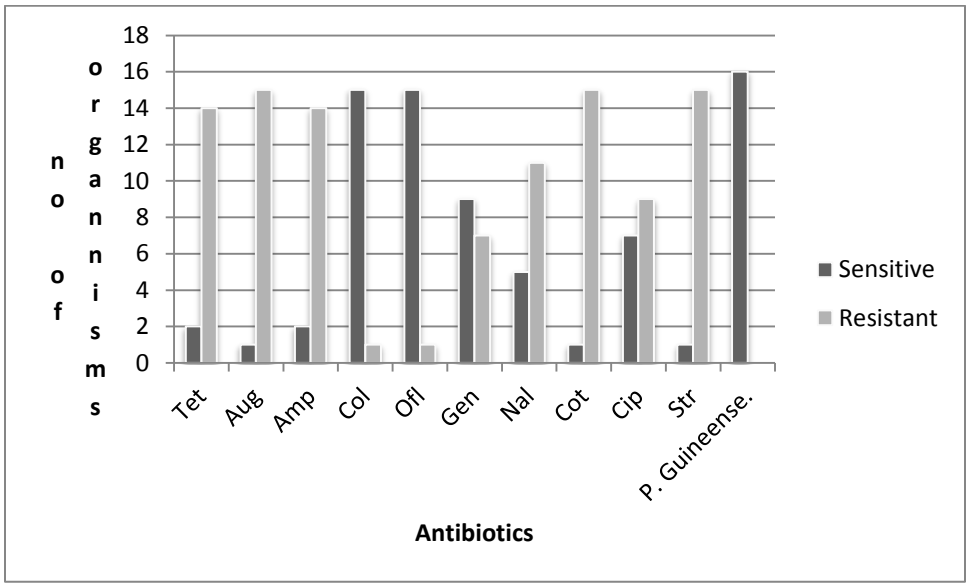

Figure1: Number of organisms showing resistance compared with the number of organism sensitive to the different antibiotics.

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