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RESEARCH ARTICLE

BACTERICIDAL AND BACTERIOSTATIC POTENCY OF SOME PHYTO-BACTERICIDES AGAINST SELECTED ESBL PRODUCING BACTERIA.

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Abstract

The study to investigate the bactericidal and bacteriostatic potency of some phyto-bactericides against selected ESBL producing bacteria was carried out. Methanolic leaf extracts of *Chromoleana odorata*, *Lasianthera ofricana*, *Heinsia crinata*, *Piper guineense*, *Aspilia africana* and *Lasianthera afrciana* were tested against ESBL producing *E. coli* and *K. pneumonia* isolated from clinical specimens (urine, wound swabs, stool, blood, high vaginal swab and sputum obtained from hospitals in five of the six states of the South-South geo-political zones of Nigeria namely; Akwa-Ibom, Cross-River, Delta, Edo and Rivers States) using both the agar disc diffusion and agar well diffusion method. Results obtained from the study revealed that all the plant extracts showed varying degree of antibacterial potency against the ESBL producing *E.coli* and *K. pneumonia*. The zones of inhibition increased with a corresponding increase in the concentration of the plant leaf extracts, with higher zones of inhibition observed at 300 mg/ml (13.2±0.25 and 20.4±0.11mm (*C. odorata*); 17.1±0.09 and 18.3±0.25mm (*A. Africana*); 19.8±0.73 and 10.8±0.25mm (*H. crinata*); 19.2±0.39 and 24.3±0.39mm (*P. guineense*); 15.5±0.42 and 18.5±0.03mm (*L. africana*) as against the ESBL producing *E. coli* and *K. pneumonia* respectively). Although, *P. guineense* showed the least minimum inhibitory concentration (50mg/ml) against the isolates, however the MIC of the plant extracts recorded were higher than that observed with the conventional antibiotic gentamicin (1.5mg/ml). The study has revealed that the phyto- constituents of *C. odorata*, *A. africana*, *H. crinata*, *P. guineense* and *L. africana*, have high antimicrobial property as compared and confirmed with commercial antibiogram. Hence, these plants could serve as an alternative medicine without side effects and in addition they could further be used to discover other bioactive natural products that may serve as lead for the development of new phyto-pharmaceuticals.

Introduction:-

ESBLs are known as extended spectrum because they are able to hydrolyze a broader spectrum of β-lactam antibiotics than the simple parent β-lactamases from which they are derived (Andy et al., 2019). They have ability to

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even inactivate β-lactam antibiotics containing an oxyimino-group such as oxyimino- cephalosporin as well as oxyimino-monobactam (Ikegbunam et al., 2014). They are not active against cephamycins and carbapenems. Generally, they are inhibited by β-lactamase- inhibitors such as clavulanate and tazobactem (Muza heed et al., 2008). ESBLs have been found in a wide range of gram-negative rods. However, the vast majority of species expressing these enzymes belong to the family Enterobacteriaceae (Kiratisin et al., 2007).

Medicinal plants provide basic raw materials for different industries such as pharmaceutical, cosmetic, perfumery and food (Varalakshmi et al, 2014). The medicinal plants are referred to as plants that are used for their therapeutic or medicinal values (Mukesh et al., 2018). The whole plant or its different parts may be valued for its therapeutic, medicinal, aromatic or savory qualities (Sahid et al., 2003). They also play vital role as an antimicrobial agent. There has been resurgence in the consummation and demand for medicinal plants. Many plants components are now synthesized in large laboratories for their use in pharmaceutical preparations. A wide range of secondary metabolites are produced by plants which can be directly used as precursors or as principal compound for drug synthesis in pharmaceutical industries (Agbafor et al., 2011). It is anticipated that plant extracts exhibiting target sites other than those used by antibiotics can be effective against microbial pathogens which are drug resistant. Bioactive compounds are normally accumulated as secondary metabolites in all plant cell but their concentration is determined by the plant part, climate and growth phase (Dhiman et al., 2011). The highest concentration of such compounds is present in leaves generally preferred for therapeutic use (Dhiman et al., 2011).

The antimicrobial activity of extracts of various plants has been proven scientifically. These extracts contain multiple active ingredients which could deter the development of resistance. Currently, there are few medicinal plants that have been established to have antimicrobial activity against ESBL producing organisms, hence the need for this present study.

Material And Methods:-

Test microorganism

The test organism used in this study were ESBL producing bacteria isolates obtained from various clinical specimens (urine, wound swab, stool, blood, high vaginal swab and sputum) from two hospitals in five out of the six states of the South-South geopolitical zones namely; Akwa-Ibom (University of Uyo Teaching Hospital and St. Luke's Hospital Anua in Uyo), Cross River (University of Calabar Teaching Hospital and General Hospital, Calabar), Delta (Federal Central Hospital Asaba and NNPC Clinic, Warri), Edo (University of Benin Teaching Hospital and Central Specialist Hospital, Benin) and Rivers (University of Port Hartcourt Teaching Hospital and Brait Waite-Memorial Hospital, Port Hartcourt).

Herbal extract

The plants used for this study were Chromoleana odorata, Aspilia africana, Heinsia crinata, Piper guineense and Lasianthera africana. They were identified and authenticated by a taxonomist in the Department of Plant and Ecological studies, University of Calabar, and voucher specimens were deposited there. The leaf parts were air-dried at room temperature and reduced into fine powders using a mechanical blender. 100g of the powdered materials were extracted using 100ml analytical grade methanol (BHD laboratory, England) via cold maceration for forty - eight hours. The resulting mixture was filtered and the filtrate was concentrated using a Rotary evaporator (Model RE 300, Barloworld Scientific Ltd, UK) and kept at room temperature for the methanol to completely evaporate for 24hrs. The resulting residue which was the methanol leaf extract of the various plants was stored in air-tight containers.

Plate 1:-Lasianthera africana

Plate 2:-Aspilia Africana

Plate 3:-Heinsia crinata

Plate 4:-Piper guineense

Plate 5:-Chromolaena odorata

Standard stock solutions

Standard stock solutions of 50mg/ml, 100mg/ml, 150mg/ml, 200mg/ml, 250mg/ml and 300mg/ml of Chromoleana odorata, Aspilia africana, Heinsia crinata, Piper guineense and Lasianthera africana leaves respectively, were prepared in dimethylsulphoxide (DMSO, BDH-Laboratory, England). Two-fold serial dilutions of the stock solutions were prepared while carrying out the various tests. 100µg/ml stock solution of gentamicin was diluted to obtain concentrations of 0.5mg/ml, 1.0mg/ml, 1.5mg/ml, 2.0mg/ml and 2.5mg/ml, which were used in this study.

Sensitivity of ESBL producing organisms to the plant extracts and standard antibiotics.

This was determined by using the disc diffusion method for the standard antibiotics and agar well diffusion method for the plant extracts (CLSI, 2012). Briefly, a Petri-dish was divided into five sections; one section for the stock solution and each dilution of a plant extract in DMSO. 0.1ml of the standardized suspension of the isolates were put into the empty sterile petri-dish. Bijou bottles containing 20ml of sterile molten Mueller- Hinton agar at 45^0C was poured into each of the plates containing the suspension of the isolate. These were gently rotated thoroughly and were allowed to set for 20 minutes. Six millimeters (6mm) cork borer dimension was used to boreholes into each section in the plates and each section was labeled properly. About 40µl of the various concentrations of the extracts were placed into the wells and left for one hour at room temperature. The plates were incubated at 37^0C for 18-24 hours. The test was carried out in triplicates for each isolates. The experiment was carried out with all the plant extracts. The experiment was repeated using the standard antibiotics disc (oxoid). Ceftriaxone (30µg), Chloramphenicol (30µg), Ciprofloxacin (30µg), Amoxicillin (30µg), Gentamicin (30µg), Imipenem (30µg), and

Tetracycline (30µg) were placed on each section of the plate, up to a maximum of five sections. After the incubation period, the plates were observed and inhibition zone diameters (IZD) were measured.

Evaluation of the minimum Inhibitory Concentration (MIC) of the plant extract

The minimum inhibitory concentration of the extracts against E. coli and K. pneumonia expressing ESBL were performed using the agar dilution method (Chah et al., 2006). 19 mls of sterilized molten nutrient agar was aseptically poured into sterile petri-dishes containing 1ml of the graded concentrations of the extracts. The plates were rotated to ensure even distribution of the plant extracts and allowed to set. The plates were swabbed with 0.05ml of standard suspension of the ESBL producing E. coli and K. pneumonia isolates. The plates were incubated at 37^0c for 18-24hours. The presence of growth was observed after incubation.

Results:-

Antibiogram of ESBL producing E.coli and K. pneumonia isolates

The antibiogram of the ESBL producing E. coli and K. pneumonia isolates is shown in Table 1 below. The isolates were multi- drug resistant. They were resistant to amoxicillin and chloramphenicol but were sensitive to ciprofloxacin, gentamicin, ceftriaxone, erythromycin, tetracycline and imipenem.

Sensitivity of the ESBL producing E. coli and K. pneumonia to the plant extracts

Table 2 present the result of the antimicrobial activity of the methanolic leaf extract of C. odorata on ESBL producing E. coli and K. pneumonia. it showed that a higher zone of inhibition of 13.2±0.25mm and 20.4±0.27mm was recorded at 300mg/ml, when the extract was tested against E.coli and K. pneumonia respectively. Similarly, Table 3 present the result of antimicrobial activity of the methanolic leaf extract of A. Africana on ESBL producing E. coli and K. pneumonia. it showed that the zones of inhibition increased with a corresponding increase in the concentrations of the plant extract. However, a higher zone of inhibition (17.1±0.09mm and 18.3±0.25mm) was observed at 300mg/ml, when the extract was tested against ESBL producing E. coli and K. pneumonia respectively. The result of the antimicrobial activity of the methanolic leaf extract of H. crinata, P. guineense and L. Africana against ESBL producing E. coli and K. pneumonia are presented in Table 4, 5 and 6 respectively. At a concentration of 300mg/ml, a higher zones of inhibition (19.8±0.73 and 10.8±0.25mm; 19.2±0.39 and 24.3±0.39mm; 15.5±0.42 and 18.5±0.43mm) were observed when extracts of H. crinata, P. guineense and L. africana were tested against ESBL producing E. coli and K. pneumonia.

The result of the minimum inhibitory concentration of the plant extracts against the ESBL producing E. coli and K. pneumonia is presented in Table 7. It showed that P. guineense had the lowest minimum inhibitory concentration (50mg/ml) as compared to that observed with the other extracts tested against the ESBL producing E. coli and K. pneumonia.

Table 1:-Antibiogram of ESBL producing E.coli and K. pneumonia before treatment with methanolic leaf extracts

	Inhibition zone diameter of Antibiotics (mm)							
Isolate	**CHL**	**AMX**	**CIP**	**GEN**	**CEF**	**ERY**	**TET**	**IMP**
E. coli	0	0	14	13	12	13	18	29
K. pneumonia	0	5	16	15	14	16	20	31

Keys: CHL- Chloramphenicol, AMX- Amoxicillin, CIP- Ciprofloxacin, GEN- Gentamicin, CEF- Ceftriaxone, ERY – Erythromycin, TET- Tetracycline, IMP- Imipenem

Table 2:-Antimicrobial activity of the methanolic leaf extract of C. odorata on ESBL producing E. coli and K. pneumonia

Zones of inhibition (mm)		
Concentration (mg/ml)	**E. coli**	**K. pneumonia**
50	3.1±0.18	6.4±0.19
100	5.3±0.19	8.6±0.21
150	7.4±0.21	11.8±0.24
200	9.3±0.23	15.7±0.25
300	13.2±0.25	20.4±0.27

*Each value represents the mean value of two determinants

Table 3:-Antimicrobial activity of the methanolic leaf extract of A. africana on ESBL producing E. coli and K.pneumonia

Zones of inhibition (mm)		
Concentration (mg/ml)	**E. coli**	**K. penumoniae**
50	5.2±0.7	6.4±0.18
100	7.8±0.06	9.2±0.19
150	11.5±0.06	12.8±0.20
200	14.3±0.08	15.3±0.24
300	17.1±0.09	18.3±0.25

*Each value represents the mean value of two determinants

Table 4:-Antimicrobial activity of the methanolic leaf extract of H. crinata on ESBL producing E. coli and K. pneumonia

Zones of inhibition (mm)		
Concentration (mg/ml)	**E. coli**	**K. penumoniae**
50	4.7±0.16	2.1±0.18
100	7.9±0.21	3.8±0.21
150	11.2±0.43	6.4±0.22
200	15.4±0.56	7.3±0.22
300	19.8±0.73	10.8±0.25

*Each value represents the mean value of two determinants

Table 5:-Antimicrobial activity of the methanolic leaf extract of P. guineense on ESBL producing E. coli and K. pneumonia

Zones of inhibition (mm)		
Concentration (mg/ml)	**E. coli**	**K. pneumonia**
50	4.3±0.18	6.4±0.21
100	7.4±0.24	11.8±0.28
150	11.6±0.29	14.9±0.31
200	15.3±0.31	18.6±0.32
300	19.2±0.39	24.3±0.30

*Each value represents the mean value of two determinants

Table 6:-Antimicrobial activity of the methanolic leaf extract of L. africana on ESBL producing E. coli and K. pneumonia

Zones of inhibition (mm)		
Concentration (mg/ml)	**E. coli**	**K. pneumonia**
50	4.2±0.24	5.6±0.25
100	7.3±0.28	8.4±0.29
150	9.4±0.31	12.5±0.31
200	11.6±0.39	15.9±0.38
300	15.5±0.42	18.5±0.43

*Each value represents the mean value of two determinants

Table 7:-Minimum inhibitory concentration of the plant extracts against the ESBL producing E. coli and K. pneumonia

	Minimum inhibitory concentration					
Isolates	**C. odorata (mg/ml)**	**A. africana (mg/ml)**	**H.crinata (mg/ml)**	**P.guineense (mg/ml)**	**L. africana (mg/ml)**	**Gentamicin (mg/ml)**
E. coli	100	50	50	50	100	1.5
K.pneumoniae	50	100	100	50	100	1.5

Discussion:-

In this study, the sensitivity of ESBL producing E. coli and K. pneumonia to imipenem was not surprising, as it is in accordance with previous studies by Pena et al., (1998) and Winokur et al., (2001). The resistance of the isolates to amoxicillin and chloramphenicol could be due to the co-existence of genes encoding drug resistance to other antibiotics on the plasmids which encode ESBLs (Ikegbunam et al., 2014). The methanolic leaf extracts of all the plants used (C. odorata, A. africana, H. crinata, P. guineense and L. africana) showed varying degree of antimicrobial activities against the ESBL producing E. coli and K. pneumonia tested. However, the zones of inhibition observed with the plant extracts increased with a corresponding increase in the concentrations of the extracts. This observation was not surprising as similar study by Hrindhya and Kulandhaivel (2017) reported to have recorded a high zone of inhibition when leaf extract of C. odorata was tested against pyogenic pathogens. Odinakachukwu et al., (2019) reported to have observed the antibacterial activity of leaf extract of Chromolaena odorata and the effect of its combination with some conventional antibiotics on pathogenic organisms isolated from wounds. Studies by Anibijuwon et al., (2010) and Obioma et al., (2017) reported antimicrobial activities of leaf extract of Aspilia africana on some pathogenic organisms of clinical and wound origin. Anyanwu and Nwosu (2014) and Olusimbo et al., (2011) reported antimcoribal activities of Piper guineense against some pathogens.

Amongst the plant extracts tested, P. guineense had the lowest minimum inhibitory concentration (50mg/ml) against the ESBL producing E. coli and K. pneumonia investigated. Although, Gentamycin had a far lower minimum inhibitory concentration (1.5mg/ml) against the isolates, however, all the plant extracts showed signs of antibacterial potency agent the tested isolates.

The medicinal value of a plant can be determined on the basis of bioactive compounds. Several reports by Olusimbo et al., (2011); Bassey et al., (2013); Anyanwu and Nwosu (2014) and Obioma et al., (2017) have recorded the presence of tannins, phenolic compounds,, flavonoids, terpenoids, glycosides and alkaloids in extracts of C. odorata, A. afrciana, H. crinata, P.guineense and L. africana. The presence of these phyto-components plays a vital role in the antimicrobial properties of the plants. Alkaloids are major plant components responsible for antimicrobial activity. It intercalates with DNA and interferes with the cell division (Kigigha and Zige, 2013). Flavonoids are a major group of plant phenolic compounds that act as antioxidant. It forms a complex with extracellular soluble proteins, bacterial cell walls and also disrupts the cell membrane (Divya et al., 2014). Terpenoids are a subclass of prenylipids and are naturally occurring organic chemicals which damages the cell membrane (Bassey et al., 2013). Tannins are polymeric phenolic substances which precipitate microbial protein and also inactivate microbial adhesion, enzymes and cell wall envelope transport proteins (Hridhya and Kulandhaivel, 2017).

Conclusion:-

This study on the bactericidal and bacteriostatic potency of some phyto-bactericides against selected ESBL producing bacteria has revealed that the phyto-constituents of C. odorata, A. africana, H. crinata, P. guineense and L. africana have high antimicrobial property as compared and confirmed with commercial antibiogram. Since the drug resistance nature of bacteria have been reported to be on the increase as each day passes, these plant extracts could serve as an alternative medicine without side effects. In addition, these plants could further be used to discover other bioactive natural products that may serve as lead for the development for new phyto-pharmaceuticals.

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