Bioactivity of *Bryophyllum pinnatum* and *Rauvolfia vomitoria* on Neonatal Group B *Streptococcus*

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Research Article

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Abstract

Background

Group B *Streptococcus* (GBS) is a harmless commensal bacterium in healthy adults but it causes sepsis in neonates resulting in a high rate of mortality. This study was carried out to investigate the antibacterial activity of *Bryophyllum pinnatum* and *Rauvolfia vomitoria* bioactive fractions on thirty-five multidrug resistant Group B *Streptococcus* (GBS) strains implicated on neonatal sepsis as well as to identify the antibiotic resistant genes present. The phytochemical analyses were carried out using column chromatography. Antibacterial activity, Minimum Inhibitory Concentration (MIC) and Minimum Bacteria Concentration (MBC) of the different plant biofractions was determined using disc diffusion technique. The presence of erythromycin (*erm*B) and tetracycline (*tet*O) resistant genes was identified using duplex PCR techniques. Graphing was done using Graph pad prism (version 5.0a).

Result

The results obtained from this study show that the selected plants have dose dependent activity against GBS. Ethanol biofraction of *R. vomitoria* had the highest activity with an MIC value ranges between 12.5 mg/ml and 50 mg/ml and MBC ranges from 25 mg/ml and 50 mg/ml followed by methanol biofraction of *B. pinnatum* with an MIC value ranging between 50mg/ml and 100mg/ml on 32 out of the 35 strains investigated, leaving the other three having MIC values of 25 mg/ml then MBC values ranging between 50 mg/ml and 100mg/ml while N-hexane and aqueous biofractions had the least activity. Also, the presence of *erm*B and *tet*O resistant genes were present in all the ten representative GBS strains tested.

Conclusion

The high rate of activity shown by the methanol and ethanol extracts of both plants suggests that the plants can serve as a potential alternative for the treatment of neonatal sepsis. However, a further study on their *in vivo* activity is important in order to evaluate the efficiency, safety and potential adverse effects and drug herb interactions of the plants.

Introduction

Group B *Streptococcus* (GBS) is a harmless commensal bacterium colonizing up to 30% of healthy adults but can cause severe invasive infections especially in newborn [1]. Multidrug resistant GBS is responsible for a wide range of infections in newborns including neonatal sepsis, meningitis and pneumonia [2]. GBS is among the leading cause of sepsis in neonates with mortality rate of 28% [3]. Studies in some Sub-Saharan African countries like Zimbabwe, Malawi, Kenya and Gambia reveled GBS as emerging main cause of neonatal sepsis [4]. The recent report documented by [5] indicated that 40% of neonatal sepsis cases are due to resistant pathogens. Resistant strains among several groups of microorganisms have also been widely reported; with the major predisposing factor being the constant and overuse of antibiotics [6]. Resistance to major antibiotics is mediated by genes in the microorganism that acts as efflux pump to pump out the active compounds in the antibiotics once it is been taken or genes that protect the target sites before the antibiotics could carry out its effects on the organisms [7]. The presence of the genes has made treatments very difficult and nearly impossible in the most parts of the world [8]. Hence, in view of the aforementioned there is a need to search for an alternative and sustainable, natural, safer and ecofriendly alternative drug from phytochemicals in plants that could be applied in the management of resistant clinical isolates [9]. Medicinal plants are considered the new alternative because they contain a wide range of pharmacological active compounds that can be used in the treatment of numerous chronic infectious diseases. This might be linked to their cost effectiveness, with minimal side effect [10]. In western part of Nigeria, many different indigenous plants such as *Bryophyllum pinnatum*, *Azadirachta indica*, *Rauvolfia vomitoria, Mangifera indica*, *Allium cepa*, *Mormodica charantia* among others are used for the treatment of infant diseases[11].There is therefore need for scientific validation to the therapeutic claim of indigenes by exploring the antibacterial effects of *Bryophyllum pinnatum* and *Rauvolfia vomitoria* against GBS causing neonatal sepsis [12]. This study reports the antibacterial activity of *Bryophyllum pinnatum* and *Rauvolfia vomitoria* against GBS strains causing neonatal sepsis and identification of *tet*(O) and *erm*(B) genes on same strains.

Methods

SAMPLE COLLECTION AND STRAIN CONFIRMATION

The thirty-five GBS strains used in this study were clinical isolates of GBS all resistant to both tetracycline (MIC, ≥ 8µg/ml) and erythromycin (MIC, ≥1µg/ml) from blood of neonates presenting with symptoms of sepsis according to WHO case definition of neonatal septicaemia. The strains were confirmed using standard bacteriological and biochemical screening such as CAMP test, Bacitracin test, sugar fermentation test. All9 GBS isolates were tested for multidrug resistance using some of the conventional antibiotics from the WHO model lists of essential medicine for children [13].

PLANTS MATERIALS AND PREPARATION OF BIOFRACTIONS

The two medicinal plants, *Bryophyllum pinnatum* and *Rauvolfia vomitoria* were collected from Oye-Ekiti, Ekiti State (Latitude 7.78N and Longitude 5.32E). The plants were taxonomically identified and authenticated at the herbarium of the department of Botany, Ekiti State University with the voucher number UHAE 2019/819 and UHAE 2019/814 respectively. The voucher specimens were deposited there for further references. The plant samples were air-dried for 3 weeks and pulverized to fine powdered using industrial blender (pharma model number 001). Fifty grams of powdered samples of each plant was extracted with ethanol, methanol, n-hexane and water using a soxhlet extractor at 60^{o}C-100^{o}C for 4-8hours [14]. The extracts were concentrated using Rotary Evaporator (SENCO Technology Co. Ltd. Model No: W2-1005). The crude extracts were stored in sterile McCartney bottles and refrigerated at 4^{o}C. The stock solutions were then diluted with 5% DMSO to get three varied concentrations (25mg/ml, 50mg/ml and 100mg/ml). The extracts were tested for sterility using Millipore filtration. Column chromatography of the different concentrations of stock solution of different medicinal plants was done using different solvents (ethanol, methanol, n-hexane and water) and the separated bioactive fractions of different polarities were collected. Microbe-free proof of bioactive fractions was done according to [15].

QUALITATIVE AND QUANTITATIVE PHYTOCHEMICAL SCREENING

Phytochemical screening was carried out on the ethanol crude extracts of the selected medicinal plants using standard methods as described by [16, 17]. Phytochemical screening reveals the presence of saponin, cyanide, tannin, phytate, oxalate and alkaloids. Total alkaloid contents of the ethanol extracts of both plants were determined using [18] with slight modifications. The total oxalate and phytate content were determined by adopting the method described by [19]. Total tannin content was determined using the Folin-Dennis specrophotometric method as described by [20]. The determination of the total cyanide content and saponin content was carried out using the alkaline titration method of Association of Official Analytical chemists [21] and the method described by [22].

ANTIBIOTICS SUSCEPTIBILIY PATTERN

Kirby - Bauer disc diffusion method was used to screen for antibiotic susceptibility test using the method described by [23] with slight modification. All tests were performed in triplicates and the antibacterial activity was expressed as mean diameter zones (mm) produced by the antibiotics.

ANTIBACTERIAL SUSCEPTIBLITY TESTING ON THE BIOACTIVE FRACTIONS

Antibacterial activity was screened using the disc diffusion assay [24]. The GBS strains was first adjusted to 0.5 McFarland standards and then inoculated uniformly onto the surface of sterile Mueller Hinton agar using sterile swab sticks. Sterile filter paper disc (whatman no 1, England, 6mm diameter) were impregnated/prewetted with 10µl of the biofraction prepared at a concentration of 25mg/ml, 50mg/ml and 100mg/ml and then placed on the inoculated plates at an appropriate distance from each other. Sterile filter paper discs impregnated with DMSO was used as the negative control and amoxicillin 50mg/ml was used as positive control. All plates were left for 30min at room temperature to allow the absorption of the fraction and then incubated at 37^{o}C for 24 h. After the incubation period, the mean diameter of inhibition halo was measured in millimeters [25]. Depending on the size of the zone and adopted assessment criteria according to [26] microorganisms were defined as sensitive or resistant. The Minimum Inhibitory Concentration (MIC) was carried out using a 5-fold serial dilution of the bioactive fraction stock with the highest activity (100mg/ml) bringing the final concentration to 100mg/ml,50mg/ml, 25mg/ml, 12.5mg/ml and 6.25mg/ml. The lowest concentration without visible growth was reported as MIC. The MBC was tested by pour plating the tube content without visible

growth onto sterile Mueller Hinton agar plates and then incubated at 37^{o}C for 48 h. MBCs were recorded as the lowest concentration of extracts that did not yield any growth or yielded less than ten pure colonies after incubation period. The MIC and MBC values were determined in duplicate.

MOLECULAR IDENTIFICATION OF *TET*(O) AND *ERM*(B) GENES

A duplex polymerase chain reaction (PCR) technique of [4] was used to identify the erythromycin and tetracycline resistant genes on ten representative strains using primers specific for *Streptococcus agalactiae tet*(O) *and erm*(B) (table 1), at Molecular and Biotechnology Laboratory of Federal University of Technology (FUTA), Akure, Nigeria. Ribonucleic acid (RNA) was isolated manually from the bacteria cells in an agar using total RNA purification kit (NorgenBiotek Corp, Thoroid , Canada). The lysis of the bacteria cells was performed using lysozyme-containing TE buffer [3 mg/ml lysozyme in (50 mMTris base, 10 mM EDTA buffer; pH 7.4). The concentration and purity of isolated RNA was determined at $A_{260\ nm}$ and A_{260}/A_{280} nm absorption.

Complementary deoxyribonucleic Acid (cDNA) Synthesis was done using FIRE Script RT cDNA Synthesis kit from Solis BioDyne (Tartus, Estonia) in a 3-step reaction condition: 25^{o}C for 10 min, 42^{o}C for 30 min and 85^{o}C for 5 min. The synthesized cDNA was stored at -20^{o}C for further downstream application. Primers specific for *Streptococcus agalactiae etet*(O) *and erm*(B) resistant genes were used. Strains were screened for the detection of resistance genes *tet*(O) *and erm*(B) using Polymerase Chain Reaction (PCR). The selection of the strains was based on the antibiotic susceptibility profiles. Polymerase Chain Reaction (PCR) for the amplification of gene of interest was performed using 5x FIREPol® Master Mix kit from Solis BioDyne (Tartus, Estonia) in a thermocycler (Primer 96 PCR- system MWG genomic Technology) according to manufacturer's protocol.

The PCR conditions used are as follows; 95^{o}C for 5 minutes (initial denaturation), 40 cycles of 95^{o}C for 30 seconds (denaturation), 56^{o}C for 30 seconds (annealing), 72^{o}C for 1 minute (extension) and 1 cycle of 72^{o}C for 10 minutes (final extension) followed by cooling and holding at 4^{o}C respectively. To check whether the PCR generated the anticipated DNA fragment (amplicon), agarose gel electrophoresis was employed for size separation for the PCR products.

Table 1: Primers for PCR assay

S/N	Gene	Sequence	Start	Length	Tm	GC%	Amplicon Size	Genebank Accession Number
1	Str-aga-*erm*B	Forward: CCGTGCGTCTGACATCTATC	293	20	61.904	55	202	EF422365.1
		Reverse: ATCTGGAACATCTGTGGTATGG	494	22	61.813	45.455		
2	Str-aga-*tet*O	Forward: GAACAGTGGGATGCGGTAAT	677	20	62.229	50	221	EF472563.1
		Reverse: CCTTCAGGCCGTTGATGAATA	897	21	62.245	47.619		

Statistical Analysis

Results calculated from replicate data were expressed as means±standard error (SEM). Graphing was performed using Graph Pad Prism (ver.5.0a).

Results

PHYTOCHEMICAL ANALYSIS

The results of the phytochemical analysis revealed the presence of saponin, alkaloid, phytate, oxalate, cyanide and tannin in the both medicinal plant ethanol extracts. Quantitative analysis of these phytochemical is recorded as shown in table 2.

ANTIBIOTIC SUSCEPTIBILITY PROFILE

There were different levels of resistance among the different antibiotics with the following frequencies as shown in table 3; ampicillin (AMP) 94.2% (33 of 35), tetracycline (TET) 85.7% (30 of 35), vancomicin (VAN) 74.2% (26 of 35), Gentamicin (GEN) 11.4% (4 of 35), cefuroxime (CRX) 97.1% (34 of 35) and erythromycin (ERM) 85.7% (30 of 35). Antibiogram pattern of multiple antibiotics resistance among GBS strains were represented in table 4. Here, 20 (57%) strains were simultaneously resistant to erythromycin and tetracycline antibiotics (cross resistance). At least, 22 (62.8%) strains were resistant to two of the different classes of antibiotics with only four (11.4%) resistant to both erythromycin and Gentamicin. At least 21 out of 35 (60%) GBS strains were resistant to 3 antibiotics out of the 6 antibiotics used. At least 20 (57.1%) were resistant to four antibiotics used with cross resistance between ERY,TET,VAN,GEN having just 4 (11.4%) strains resistant to all of the four antibiotics. At least 20 (57%) were resistant to five antibiotics used with cross resistance between ERY,TET,VAN,GEN, AMP having just 4 (11.4%) strains resistant to all of the five antibiotics (31.4%). More so, 4 out of 35(11.4%) GBS strains were resistant to all of the six antibiotics.

ANTIBACTERIAL SUSCEPTIBILITY PATTERN OF BIOACTIVE FRACTIONS AGAINST GBS

The results of the antibacterial activity revealed a dose dependent antibacterial activity amongst all the different solvents biofractions (figure 1). There was a comparable antibacterial activity of most of the different solvent biofractions with the conventional antibiotics and in some cases a better activity compared with the conventional antibiotics. The antibacterial activity of *Bryophyllum pinnatum* and *Rauvolfia vomitoria* solvent biofractions gave the mean zones of inhibition to be (21±2.0mm,19±3.6mm) for methanol, (20±3.6mm,23±1.0mm) for ethanol, (20±3.6mm,19±2.6mm) for N-hexane and (15±2.0mm,17±3.6mm) for aqueous. The MIC and MBC values (table 5) obtained showed that ethanol biofraction of *Rauvolfia vomitoria* had the highest activity with an MIC ranging between 12.5 mg/ml and 50 mg/ml and MBC ranging between 25 mg/ml and 50 mg/ml followed by methanol biofraction of *Bryophyllum pinnatum* with an MIC ranging between 50mg/ml and 100mg/ml on 32 out of the 35 strains investigated, leaving the other three having MIC values of 25 mg/ml then MBC values ranging 50 mg/ml between and 100 mg/ml.

MOLECULAR IDENTIFICATION OF *TET*(O) AND *ERM*(B) GENES

Ten representative clinical strains of *Streptococcus agalactiae* resistant to both erythromycin (MICs ≥ 1 µg/ml) and tetracycline (MICs ≥ 2 µg/ml) were genotyped for erythromycin and tetracycline resistance genes using primers specific for *Streptococcus agalactiae tet*(O) *and erm*(B). We found all of the 10 GBS strains having the *erm*(B) and the *tet*(O) genes present (Figure 2 and Figure 3); this could imply that these resistant genes are one of the possible causes of antibiotic resistance among these strains.

Table 2: Qualitative and quantitative phytochemical analysis of *Rauvolfia vomitoria* and *Bryophyllum pinnatum*

Sample	Alkaloids (%)	Oxalate (%)	PhytateMol/Kg	Saponin (%)	Tannin (%)	Cyanide Mol/Kg
Bryophyllum pinnatum	14.50	0.036	0.090	12.86	20.595	2.700
Rauvolfia vomitoria	17.88	0.022	0.039	19.60	12.815	8.316

Table 3: FREQUENCY OF ANTIBIOTIC SUSCEPTIBILITY TEST

Antibiotics	Number resistant (n=35)	Percentage resistance (%)
CRX	34	97.1
GEN	4	11.4
VAN	26	74.2
AMP	33	94.2
TET	30	85.7
ERY	30	85.7

KEY:

CRX=Cefuroxime GEN=Gentamicin VAN=Vancomycin AMP=Ampicillin TET=Tetracycline ERY=Erythromycin

Table 4: ANTIBIOGRAM PATTERN OF MULTIPLE ANTIBIOTICS RESISTANCE AMONG GBS STRAINS

GROUP	RESISTANT ANTIBIOTICS	MULTIPLICITY	NUM OF RESISTANT STRAIN (n=35)
I	ERY	1	30
II	TET	1	30
III	AMP	1	33
IV	CRX	1	34
V	GEN	1	4
VI	VAN	1	26
VII	ERY TET	2	29
VIII	ERY AMP	2	29
IX	ERY CRX	2	28
X	ERY GEN	2	4
XI	ERY VAN	2	22
XII	ERY TET VAN	3	21
XIII	ERY TET GEN	3	3
XIV	ERY TET CRX	3	26
XV	ERY TET AMP	3	28
XVI	ERY TET VAN AMP GEN	5	4
XVII	ERY TET VAN AMP CRX	5	20
XVIII	ERY TET VAN AMP	4	21
XIX	ERY TET VAN CRX	4	20
XX	ERY TET VAN GEN	4	3
XXI	ERY TET AMP CRX GEN VAN	6	4

KEY:

CRX=Cefuroxime GEN=Gentamicin VAN=Vancomycin AMP=Ampicillin TET=Tetracycline ERY=Erythromycin

Table 5: THE MIC AND MBC OF METHANOLIC AND ETHANOLIC BIOFRACTIONS OF *B. PINNATUM* AND *R. VOMITORIA* BIOFRACTIONS

GBS strains	M100B (MIC) mg/ml	M100B (MBC) mg/ml	E100R (MIC) mg/ml	E100R (MBC) mg/ml
BAS19A	100	100	50	-
BAS9^{2}A	100	-	50	-
BAS9^{2}A*	100	-	50	-
BAS11A	50	100	25	-
BAS13A	100	100	12.5	25
I0.3mg/ml	50	-	50	25
BAS16An	50	100	50	-
BAS7An	100	-	25	50
BAS20^{2}An	50	100	50	-
BAS3A	25	50	12.5	25
BAS21^{2}An	100	-	25	50
BAS20An	50	100	50	-
MSAS6^{2}A	50	100	25	50
BAS20^{2}An	25	50	12.5	50
BAS24A	100	-	25	50
MSAS9A	100	-	25	50
MSAS1A	100	-	50	-
BAS22^{2}An	100	-	50	50
MACS21A	50	100	25	50
MACS1ax	50	100	12.5	25
MACS20^{2}A	50	100	50	-
MSAS7A	100	100	50	-
MSAS13A	100	-	25	50
MSAS20^{2}A	50	50	25	50
MSAS7^{2}A	100	100	20	25
MSA21^{2}A	25	50	50	-
MSAS8^{2}A	50	100	25	50
MSAS5^{2}A	100	-	12.5	25
MSAS7An	50	100	50	-
MSAS^{2}Pink	50	100	25	50
MSAS4^{2}A	50	100	25	50
MACS7^{2}A	100	-	50	50

MACS5^{2}A	100	-	50	-
BAS8^{2}A	50	50	25	50
BAS5A	50	50	12.5	25

Discussion

In this study, the results of the phytochemical analysis of *Bryophyllum pinnatum* and *Rauvolfia vomitoria* showed the presence of Alkaloid (14.50, 17.88) %, Oxalate (0.036, 0.022) %, phylate (0.090, 0.039) Mol/Kg, Saponin (12.86, 19.60) %, Tannin (20.595, 12.815) %, and Cyanide (2.7, 8.316) Mol/Kg respectively. This is in line with the reports of [27] and [28] who reported the presence of saponin, tannin, alkaloids in the stem and leaf extracts of *Bryophyllum pinnatum*. This is also in line with the works of [29, 30] but contrary to the works of [31] who reported absence of cyanide and tannin. The level of medicinal activities of most plants depends on the level of phytochemical (bioactive) constituents available in the plant. Therefore, phytochemical screening of plants is important to identify the various important compounds which may be used as bases for modern drugs discovery [32].

The susceptibility of GBS was tested against some selected broad-spectrum antibiotics (WHO model list of essential medicine for children). The resistance of the strains to the selected antibiotics was in the order CRX > AMP > TET/ERY > VAN > GEN. The results reveal a high rate of multidrug resistance by the strains, as they were highly resistant to five out of six tested antibiotics; cefuroxime 97.1% (34 of 35), ampicillin 94.2% (33 of 35), tetracycline and erythromycin 85.7% (30 of 35) each, vancomycin 74.2% (26 of 35) and Gentamycin 11.4% (4 of 35). This study is in line with the reports of [33]who reported 96% resistance to tetracycline in Iran;[34] who recorded 97.3% resistance to tetracycline by GBS in Tunishia; and [35] also recorded a high rate of resistance of *S; agalactiae* isolates obtained from urine samples to tetracycline (96%) and macrolide (35%) but the contrary to the reports of [33]who reported lesser percentage of erythromycin resistance of 8.1% to GBS isolates and 100% susceptibility to vancomycin antibiotics, [36] who detected 21.2% GBS isolates to be resistant to erythromycin; and [37] who reported low rate of resistance to erythromycin (6.5%).

Occurrence of resistant GBS isolates to the beta-lactam antibiotics, macrolides and vancomycin in the low-income countries including Africa is possibly because of the widespread use of the antibiotics empirically for the treatment of different infectious diseases and the availability of these drugs non-restrictively in different areas with lower price, enable self-prescription [38]. Obtaining data on antibacterial susceptibility is essential to optimize treatment and minimize the emergence of bacterial resistance, which is responsible for the increasing number of therapeutic failures presently.

Furthermore, the maximum activity of *Bryophyllum pinnatum* was obtained from the leaf extract using methanol biofraction containing 100mg/ml concentration followed by ethanol biofraction with concentration of 100mg/ml. This was in accordance with the reports of [39, 40, 41, 42] but partially in consonance to the report of [43] who reported the effectiveness of the ethanol extract derived from *Bryophyllum pinnatum* plant by exhibiting the best biological activities at 4 mm for *Staphylococcus aureus*. Meanwhile, the activity of *Rauvolfia vomitoria* leaf was discovered to be at maximum using ethanol biofraction at 100mg/ml followed by methanol biofraction at 100mg/ among the four solvents biofractions used against GBS with mean inhibition diameter ranging from (15mm-23mm). This was in accordance with the reports of [44, 45] but partially contrary to the report of [46]who reported methanol extract having the best activity against GBS. Aqueous biofractions and N-hexane biofractions of *Bryophyllum pinnatum* and *Rauvolfia vomitoria* had lesser activity on the test strains with mean inhibition diameter of (6mm-15mm,7mm-20mm) for aqueous and (10mm-17mm,10mm-19mm.) for N-hexane respectively. This less activity encountered with aqueous and N-hexane biofractions could be because the solvents did not extract all the active antimicrobial agents against GBS. Testing of the leaves of the two plants *Bryophyllum pinnatum* and *Rauvolfia vomitoria* according to the results obtained indicates that both plants have a dose dependent antimicrobial activity against GBS at different levels.

The MIC and MBC values indicates that at different concentrations, both *Bryophyllum pinnatum* and *Rauvolfia vomitoria* exhibited inhibitory and bactericidal effects on different GBS strains tested which is line with the reports of [27, 42] who reported that *B. pinnatum* has antimicrobial activity against many infectious microorganisms and [44, 45] who reported that *R. vomitoria* has some bactericidal effects against some range of bacteria.

Our study is the first prevalence report of detection of GBS antibiotic (Erythromycin and tetracycline) resistant genes in neonates in Ekiti State, Nigeria. Twenty out of thirty-five (85.7%) of GBS strains were concomitantly resistant to both erythromycin and tetracycline antibiotics and 10 representative samples genotyped for *erm*(B) and *tet*(O) resistant genes had these antibiotic resistant genes present (100%). this could also be as a result of the ubiquitous presence of *tet*(O) and *erm*(B) genes in opportunistic pathogens and members of the normal flora [47]. The result of this study is contrary to the reports of [48] who reported 2.4% *tet*(O) and 34.5% *erm*(B) gene in GBS. [49]who reported 3.9% *tet*(O) gene, [37] who reported total absence of *tet*(O) gene in all GBS strains tested, [35] who reported total absence of *tet*(O) gene in all of the *S. agalactiae* isolates and only 16% *erm*(B) gene present as well as [33] who reported only 2.2% *tet*(O) genes present. All these reports differ from the reports of this study probably because of differences in the rate of the underuse, overuse and inappropriate use of antibiotics and poor infection control practiced across the areas been reported. The high resistance to erythromycin and tetracycline in this study could be because the antibiotic is relatively cheap and extensively used as prophylaxis in the therapy of human infections. It is known that bacterial strains tend to be resistant to frequently used antibiotics [50].

Conclusion

In conclusion, the antibacterial activity of the leaves of *Bryophyllum pinnatum*and *Rauvolfia vomitoria* against neonatal GBS has been established in this study. GBS growth inhibition evidenced by the zones of inhibition, MIC and MBC values of the extracting solvents biofractions, were comparable to, and averagely higher than that observed with selected broad-spectrum antibiotics can be recommended as a potential inexpensive alternative of antimicrobial drugs. Antibacterial activities of the plant have been related to the presence of bioactive phytochemicals in the plant. Multi drug resistance in all but one (gentamicin) of the antibiotics used was reported in this study. The tetracycline resistance encoded by ribosome protection gene tet(O) and the ribosomal methylation encoded by erythromycin ribosomal methylase gene, erm(B) are the two genes responsible for resistance in this study as evidenced by its presence in the PCR results.

Therefore, encouraging research output in the area of medicinal plants will help reduce antibiotic drug resistance and provide cheap alternative medicine which will be made available in our various health facilities for the treatment of neonatal sepsis and other drug resistant microorganisms.

Abbreviations

(AMP) ampicillin, (cDNA) Complementary deoxyribonucleic Acid, (CRX) cefuroxime, (ERM) erythromycin, (*erm*B) erythromycin resistant gene, (GBS) Group B *Streptococcus*,(GEN) Gentamicin, (MBC) Minimum Bacteria Concentration, (MIC)Minimum Inhibitory Concentration, (PCR) Polymerase Chain Reaction,(TET) tetracycline, (*tet*O) tetracycline resistant gene, (VAN) vancomicin,(CAMP) Christie-Atkins-Munch-Peterson, (DMSO) Dimethylsulfoxide

Declarations

Authors' contribution

S.K.S.O: conceptualize the research and performed the experiment; L.U.U.: carried out the laboratory work, obtained and analyzed the data and prepared a draft of the manuscript; C.O.A: Edited the final manuscript. All authors read and approved the final manuscript.

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Competing interests

The authors declare that there are no competing interests.

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Figures

Figure 1

Antibacterial activity of *Bryophyllumpinnatum*and *Rauvolfiavomitoria* amongst all the different solvents biofractions in relation with WHO antibiotics of choice for treatment of neonatal sepsis

Figure 2

Gel image of *erm*(B) gene expression and its band intensity for 10 representative samples of GBS

Figure 3

Gel image of *tet*(O) gene expression and its band intensity for 10 representative samples of GBS