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Original Research Article

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

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ABSTRACT

Rural dwellers explore *Bryophyllumpinnatum* and *Rauvolfiavomitoria* for treating neonatal infections. The antibacterial activities of medicinal plant biofractions were evaluated against multidrug-resistant Group B *Streptococcus* (GBS) strains causing neonatal sepsis. Pulverized plant leaves were extracted with different solvents for primary extraction and subjected to column chromatography and phytochemical analysis. A broth microdilution assay to determine the minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) was performed against multidrug-resistant GBS strains. Duplex polymerase chain reaction (PCR) was employed to detect *erm*B and *tet*O-resistant genes in 10 different GBS strains that were resistant to both erythromycin and tetracycline. The data obtained was analyzed using Spearman rank correlation and ANOVA. The results obtained from this study showed that the selected plants have concentration-dependent activity against GBS. The ethanol biofraction of *R. vomitoria* was the most active, with an MIC value between 12.5 mg/mL and 50 mg/mL and an MBC value between 25 mg/mL and 50 mg/mL. It was followed by the methanol biofraction of *B. pinnatum*, which had an MIC value between 50 mg/mL and 100 mg/mL on 32 of the 35 strains that were studied. The N-hexane and aqueous biofractions were the least active. Also, *erm*B and *tet*O genes were present in all 10 GBS strains. These findings indicate that *B. pinnatum* and *R. vomitoria* could serve as potential alternatives for treating neonatal sepsis caused by GBS. This study examined the assertion of the effectiveness of medicinal herbs in treating newborn sepsis.

***Keywords*:** *Bryophyllum pinnatum*, *Rauvolfia vomitoria*, plant biofractions, phytochemicals, *erm* (B) genes, *tet* (O) genes, antibacterial, Group B *Streptococcus*

Introduction

Group B *Streptococcus* (GBS) is a harmless commensal bacterium that colonizes up to 30% of healthy adults but can cause severe invasive infections, especially in newborns. 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 causes of sepsis in neonates, with a mortality rate of 28%.[3] Studies in some Sub-Saharan African countries like Zimbabwe, Malawi, Kenya, and Gambia revealed GBS as an emerging main cause of neonatal sepsis (Akinlolu *et al*.).[4] According to a recent report by ReAct,[5] resistant pathogens account for 40% of neonatal sepsis cases. Resistance to major antibiotics is caused by genes in microorganisms that function as efflux pumps, expelling the active components of antibiotics, or genes that shield the target sites, preventing the antibiotics from exerting their effects on the organisms.[6] Therefore, it is necessary to explore alternative and sustainable drugs derived from phytochemicals in plants that are natural, safer, and environmentally friendly. These drugs could be employed to manage resistant clinical isolates.[7]

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Medicinal plants are a promising alternative as they contain a diverse array of biologically active compounds that can be utilized in the treatment of various chronic infectious diseases. This could be associated with their cost-effectiveness and minimal side effects.[8] In the western region of Nigeria, various native plants, like *Bryophyllum pinnatum*, *Azadirachta indica*, *Rauvolfia vomitoria*, *Mangifera indica*, *Allium cepa*, and *Mormodic acharantia*, are used to treat infant diseases.[9] However, it is necessary to scientifically validate the traditional medicinal claims by investigating the antibacterial properties of *Bryophyllum pinnatum* and *Rauvolfia vomitoria* against neonatal sepsis caused by Group B *Streptococcus* (GBS).[10] This study aims to assess the antibacterial effects of *Bryophyllum pinnatum* and *Rauvolfia vomitoria* against GBS strains that cause neonatal sepsis, as well as identify the presence of *tet* (O) and *erm* (B) genes in these strains.

Materials and Methods

Sample Collection and Strain Confirmation

The thirty-five GBS strains used in this study were neonatal clinical isolates of GBS, all resistant to both tetracycline (MIC, ≥ 8µg/ml) and erythromycin (MIC, ≥1µg/ml) and were obtained from the Drug Discovery and Infectious Diseases Research Group, Department of Microbiology, Federal University Oye-Ekiti, Ekiti, Nigeria. The strains were confirmed using standard bacteriological screening with 5% defibrinated sheep blood agar and biochemical screening, such as sugar fermentation tests. CAMP tests and bacitracin tests were also carried out. Thirty-five (35) GBS isolates were tested for multidrug resistance using some of the conventional antibiotics from the WHO model lists of essential medicines for children.[11]The plants were taxonomically identified and authenticated at the herbarium of the department of Botany at Ekiti State University with the voucher numbers UHAE

2019/819 and UHAE 2019/814, respectively. The voucher specimens were deposited there for further reference.

Plant Extraction

The plant samples were air-dried for 3 weeks and pulverized to a fine powder using an industrial blender (Pharma model number 001). Fifty grams of powdered samples of each plant were extracted with ethanol, methanol, n-hexane, and water using a Soxhlet extractor at 60°C-100°C for 4-8 hours.[12] The extracts were concentrated using a rotary evaporator (SENCO Technology Co. Ltd., Model No. W2-1005). The crude extracts were stored in sterile McCartney bottles and refrigerated at 4ºC. The stock solutions were then diluted with 5% DMSO to get three varied concentrations (25 mg/mL, 50 mg/mL and 100 mg/mL). The extracts were tested for sterility using millipore filtration. Column chromatography of the different concentrations of stock solutions 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 as described.[13]

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 to determine the presence of saponin, cyanide, tannin, phytate, oxalate, and alkaloids.[14,15] The determination of the total cyanide content and saponin content was carried out using the method as described.[16]

Antibiotic Susceptibility Patterns

The Kirby-Bauer disc diffusion method was used to screen for antibiotic susceptibility with a slight modification.[17] All tests were performed in triplicate, and the antibacterial activity was expressed as the mean diameter zones (mm) produced by the antibiotics.

Antibacterial Susceptibility Testing on the Bioactive Fractions

Antibacterial activity was screened using the disc diffusion assay.[18] The GBS strains were 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 discs (Whatman No. 1, England, 6 mm diameter) were impregnated/prewetted with 10µl of the biofractions obtained at a concentration of 25 mg/mL, 50 mg/mL and 100 mg/mL and then placed on the inoculated plates at an appropriate distance from each other. Sterile filter paper discs impregnated with DMSO were used as the negative control and amoxicillin 50 mg/mL was used as the positive control. All plates were left at room temperature for 30 minutes to allow the absorption of the fraction and then incubated at 37°C for 24 h. After the incubation period, the mean diameter of the inhibition halo was measured in millimeters. Depending on the size of the zone and the adopted assessment criteria, 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 (100 mg/mL), bringing the final concentration to 100 mg/mL, 50 mg/mL, 25 mg/mL, 12.5 mg/mL and 6.25 mg/mL. The lowest concentration without visible growth was reported as the MIC. The MBC was tested by plating the tube content without visible growth onto sterile Mueller Hinton agar plates and then incubated at 37°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 the incubation period. The MIC and MBC were carried out in order to precisely determine the concentration of bioactive fraction required to inhibit or stop the growth of GBS. The researchers determined the MIC and MBC values in duplicate.

PCR assay for the detection of tet(O) and erm(B) genes using duplex amplification

The duplex polymerase chain reaction (PCR) method was employed to detect the presence of erythromycin and tetracycline resistant genes in 10 out of 35 strains. This was achieved by using specific primers for *Streptococcus agalactiae tet*(O) and *erm*(B) genes (as shown in Table 1). To isolate the ribonucleic acid (RNA) from the bacterial cells, a manual extraction method was used. The cells were grown on agar and the RNA was purified using a total RNA purification kit from NorgenBiotek Corp., located in Thoroid, Canada. The bacterial cells were lysed by treating them with TE buffer containing lysozyme [3 mg/ml lysozyme in a buffer solution consisting of 50 mMTris base and 10 mM EDTA, with a pH of 7.4]. The concentration and purity of extracted RNA were assessed by measuring the absorbance at A260 nm and the A260/A280 nm ratio.

The process of synthesizing complementary deoxyribonucleic acid (cDNA) was carried out using the FIRE Script RT cDNA Synthesis Kit from Solis BioDyne, located in Tartus, Estonia. The synthesis was performed in a 3-step reaction setting, which involved incubation at 25°C for 10 minutes, followed by incubation at 42°C for 30 minutes, and finally incubation at 85°C for 5 minutes. The generated complementary DNA (cDNA) was preserved at a temperature of -20°C for subsequent utilization in downstream processes. We utilized Primers that specifically target the *tet*(O) and *erm*(B) resistance genes of *Streptococcus agalactiae* were used. Strains were examined for the presence of resistance genes *tet*(O) and *erm*(B) using Polymerase Chain Reaction (PCR). The strains were chosen based on their antibiotic susceptibility characteristics. The gene of interest was amplified using Polymerase Chain Reaction (PCR) with the 5x FIREPol® Master Mix kit from Solis BioDyne (Tartus, Estonia) in a thermocycler (Primer 96 PCR-system MWG genome technology), following the instructions provided by the manufacturer. The PCR protocol involves the following conditions: an initial denaturation step at 95°C for 5 minutes, followed by 40 cycles of denaturation at 95°C for 30 seconds, annealing at 56°C for 30 seconds, and extension at 72°C for 1 minute. Finally, there is a single cycle of final extension at 72°C for 10 minutes. The reaction is then cooled and held at 4°C. Agarose gel electrophoresis was used to verify the presence of the expected DNA fragment (amplicon) created by the PCR, by separating the PCR products based on their size.

Statistical Analysis

Means±standard error (SEM) expressed the results calculated from replicate data. Graph Pad Prism (Ver.5.0a) was used to plot the graph.

Table 1: Primers for the 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		

Results and Discussion

Biofractions from Column Chromatography

Diverse fractions were obtained from this study, which were determined by the variable concentrations employed, as indicated in Table 2. The ethanol-extracted fractions from *Rauvolfia vomitoria* at concentrations of 25 mg/mL, 50 mg/mL, and 100 mg/mL yielded fractions with values of 4, 4, and 3, respectively. Similarly, in *Bryophyllum pinnatum*, the fractions obtained at the same concentrations were 3, 3, and 4 respectively. Observations revealed that the fractions produced from *Rauvolfia vomitoria* using methanol were 3, 3, and 4 at concentrations of 25 mg/mL, 50 mg/mL, and 100 mg/mL, respectively.

Phytochemicals present in B. pinnatum and R. vomitoria

The phytochemical examination detected the presence of saponin, alkaloid, phytate, oxalate, cyanide, and tannin in both ethanol extracts of the medicinal plants. Table 3 documents the quantitative quantities of these phytochemicals. *Bryophyllum pinnatum* and *Rauvolfia vomitoria* were found to contain the following substances: 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. These findings align with the observations of Akinpelu[19] and Ozolua *et al*.[20], which documented the presence of saponin, tannin, and alkaloids in the stem and leaf extracts of *Bryophyllum pinnatum*. This also supports the findings of Ojo *et al*.[21], but contradicts the findings of Sonibare *et al*.[22], who reported the lack of cyanide and tannin. The therapeutic efficacy of most plants is contingent upon the abundance of phytochemical (bioactive) elements present in the plant. Hence, the process of analyzing plants for phytochemicals is crucial in order to uncover the diverse and significant substances that can serve as foundations for the discovery of contemporary medications.

Antibiotic Susceptibility Profile

The strains exhibited varying levels of resistance to the selected antibiotics, with the highest resistance seen for CRX, followed by AMP, TET/ERY, VAN, and GEN, as indicated in Table 4. The findings indicated a significant prevalence of multidrug resistance among the strains, as they exhibited a high level of resistance to five out of the six antibiotics tested (Table 5): cefuroxime 97.1% (34 out of 35), ampicillin 94.2% (33 out of 35), tetracycline and erythromycin 85.7% (30 out of 35) each, vancomycin 74.2% (26 out of 35), and Gentamycin 11.4% (4 out of 35). This result aligns with the findings of Hraoui *et al*.[23], who observed a 96% prevalence of tetracycline resistance in Iran. Similarly, Melo *et al*.[24] documented a 97.3% resistance rate to tetracycline by GBS in Tunisia. Additionally, Emaneini *et al*.[25] showed a significant level of resistance in S. The urine samples showed high resistance rates to tetracycline (96%) and moderate resistance rates to macrolide (35%). This is in contrast to the findings of Hraoui *et al*.[23], who reported a lower percentage (8.1%) of erythromycin resistance in GBS isolates and 100% susceptibility to vancomycin antibiotics. However, Frouhesh-Tehrani *et al*.[26] found that 21.2% of GBS isolates were resistant to erythromycin, and Elikwu *et al*.[27] reported a low rate of resistance (6.5%) to erythromycin.

The presence of drug-resistant GBS isolates in low-income countries, particularly in Africa, may be attributed to the extensive use of beta-lactam antibiotics, macrolides, and vancomycin for empirical treatment of various infectious diseases. These drugs are readily available in different areas at lower prices, allowing for self-prescription. Obtaining data on antibacterial susceptibility is crucial for improving treatment outcomes and reducing the occurrence of bacterial resistance, which is currently leading to a growing number of treatment failures.

Antibacterial Susceptibility Pattern of Bioactive Fractions against GBS

The highest level of activity of *Bryophyllum pinnatum* was achieved using a methanol biofraction of the leaf extract at a concentration of 100 mg/mL, followed by an ethanol biofraction at the same concentration (Figure 1). This finding aligns with the studies conducted by Akinsulere *et al*.[29], Ofokansi *et al*.[30], Etim *et al*.[31], and Obioma *et al*.[32]. However, it is only partially consistent with the findings of Obi and Onuoha[33], who reported that the ethanol extract derived from the *Bryophyllum pinnatum* plant showed the highest biological activity against *Staphylococcus aureus* at a concentration of 4 mm. Meanwhile, the highest activity of *Rauvolfia vomitoria* leaf was observed when employing the ethanol biofraction at a concentration of 100 mg/mL, followed by the methanol biofraction at the same concentration. These two solvents showed the most effective inhibition against GBS, with a mean inhibition diameter ranging from 15 mm to 23 mm. This finding aligns with the studies conducted by Eteng *et al.*[34] and Mokutima *et al*.[35], but it contradicts the report by Owk & Lagudu[36], who found that the methanol extract exhibited the highest activity against GBS.

Table 2: Fractions obtained from Column Chromatography

Solvent	Conc. (µg/ml)	No. of fractions – *Rauvolfiavomitoria*	No. of fractions – *Bryophyllumpinnatum*
Ethanol	25	4	3
	50	4	3
	100	3	4
Methanol	25	3	3
	50	3	4
	100	4	4
n-Hexane	25	3	2
	50	3	3
	100	2	3
Aqueous	25	3	2
	50	2	2
	100	2	2

Table 3: Qualitative and quantitative phytochemicals in *Rauvolfiavomitoria* and *Bryophyllumpinnatum*

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

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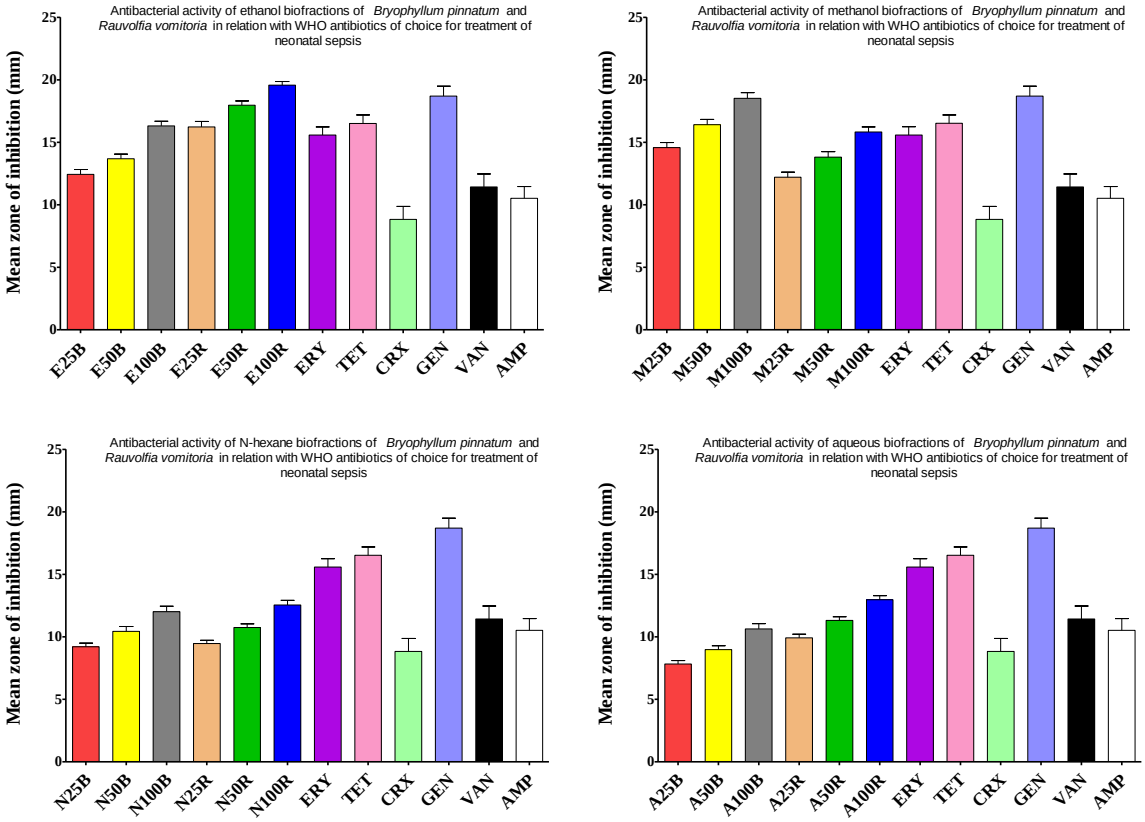

Figure 1: Antibacterial activity of *Bryophyllumpinnatum*and *Rauvolfiavomitoria* amongst all the different solvents biofractions in relation with WHO antibiotics of choice for treatment of neonatal sepsis

Table 4: Frequency of Antibiotic Susceptibility Pattern

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

The aqueous and N-hexane biofractions of *Bryophyllum pinnatum* and *Rauvolfia vomitoria* exhibited lower activity against the test strains, with mean inhibition diameters ranging from 6 mm to 15 mm and 7 mm to 20 mm for the aqueous biofractions, and 10 mm to 17 mm and 10 mm to 19 mm for the N-hexane biofractions, respectively. The reduced activity observed in the aqueous and N-hexane biofractions may be attributed to incomplete extraction of the potent antimicrobial compounds effective against GBS by the solvents used. The experimentation on the leaves of *Bryophyllum pinnatum* and *Rauvolfia vomitoria* plants demonstrates that both plants have antimicrobial activity against GBS in a dose-dependent manner, as indicated by the obtained results.

The Minimum Inhibitory Concentration and Minimum Bactericidal Concentration values presented in Table 6 demonstrate that *Bryophyllum pinnatum* and *Rauvolfia vomitoria* displayed inhibitory and bactericidal effects on various GBS (Group B *Streptococcus*) strains tested. These findings align with the observations of Akinpelu[19]and Obioma *et al*.[32], who also observed similar effects of these plants on GBS. *Bryophyllum pinnatum* exhibits antibacterial properties against numerous infectious microbes, as documented by Eteng *et al*.[34] and Mokutima *et al*.[35] who found similar findings for *R. vomitoria* exhibits bactericidal properties against a specific spectrum of microorganisms.

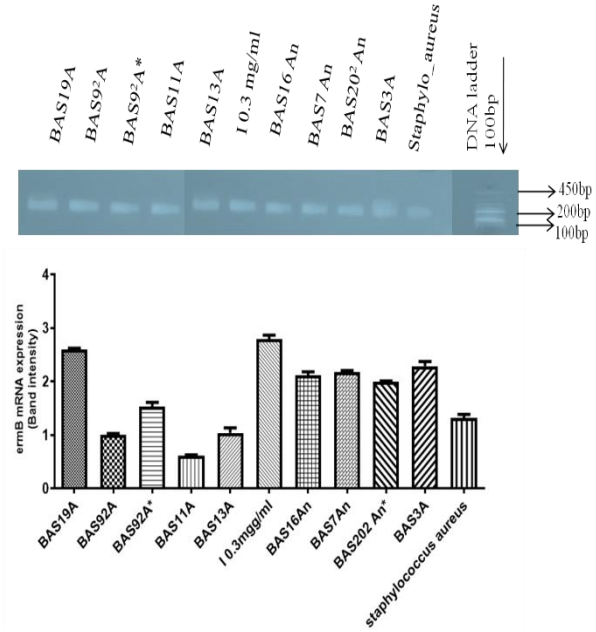

Figure 2: Gel image of *erm*(B) gene expression and its band intensity for 10 representative samples of GBS

Table 5: Antibiogram Pattern of Multiple Antibiotics Resistance among GBS Strains

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Group	Resistant antibiotics	Multiplicity	No. of Resistant strain (n=35)	Group	Resistant antibiotics	Multiplicity	No. 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 6: The MIC and MBC of Methanol and Ethanol Biofractions of *B. pinnatum* and *R. vomitoria*

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

Key: M100B – 100mg/ml methanol biofraction of *B. pinnatum*; M100R - 100mg/ml methanol biofraction of *R. vomitoria*; E100B – 100mg/ml ethanol biofraction of *B. pinnatum*; E100R - 100mg/ml ethanol biofraction of *R. vomitoria*

Antibiotic Resistant TET(O) and ERM(B) Genes in GBS Strains

This study is the first prevalence analysis of antibiotic-resistant genes for GBS (erythromycin and tetracycline) in newborns in Ekiti State, Nigeria. Out of a total of thirty-five GBS strains, twenty (85.7%) were found to be resistant to both erythromycin and tetracycline antibiotics. Additionally, all ten representative samples that were genotyped for the presence of *erm*(B) and *tet*(O) resistance genes showed 100% presence of these antibiotic resistant genes, as illustrated in Figures 2 and 3. This may also be due to the widespread occurrence of *tet*(O) and *erm*(B) genes in opportunistic pathogens and members of the normal flora.[37] However, these findings contradict the results reported by Mudzana *et al*.[38], who found 2.4% *tet*(O) and 34.5% *erm*(B) gene presence in GBS. Boswihi *et al*.[39] found that 3.9% of the GBS strains tested contained the *tet*(O) gene. Elikwu *et al*.[27], on the other hand, found that none of the GBS strains tested had the *tet*(O) gene. Similarly, Emaneini *et al*.[25] similarly found a complete lack of the *tet*(O) gene in all of the *S. agalactiae* strains tested. The *S. agalactiae* isolates showed a presence of just 16% of the *erm*(B) gene, while Hraoui *et al*.[23] reported a presence of only 2.2% of the *tet*(O) genes. The discrepancies between these reports and the findings of this investigation are likely attributable to variations in the prevalence of underuse, overuse, and improper use of antibiotics, as well as inadequate implementation of infection control measures throughout the reported regions. The observed high resistance to erythromycin and tetracycline in this study may be attributed to the widespread and cost-effective use of these antibiotics as prophylactic treatments for human illnesses. It is a well-established fact that bacterial strains have a tendency to develop resistance to commonly used antibiotics.

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, as evidenced by the zones of inhibition, MIC and MBC values of the extracting solvent biofractions, was comparable to, and averagely higher than that observed with selected broad-spectrum antibiotics and can be recommended as a potential inexpensive alternative to antimicrobial drugs. The 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 the ribosome protection gene *tet*(O) and the ribosomal methylation encoded by the erythromycin ribosomal methylase gene, *erm*(B) are the two genes responsible for resistance in this study, as evidenced by their 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.

Conflict of Interest

The authors declare no conflict of interest.

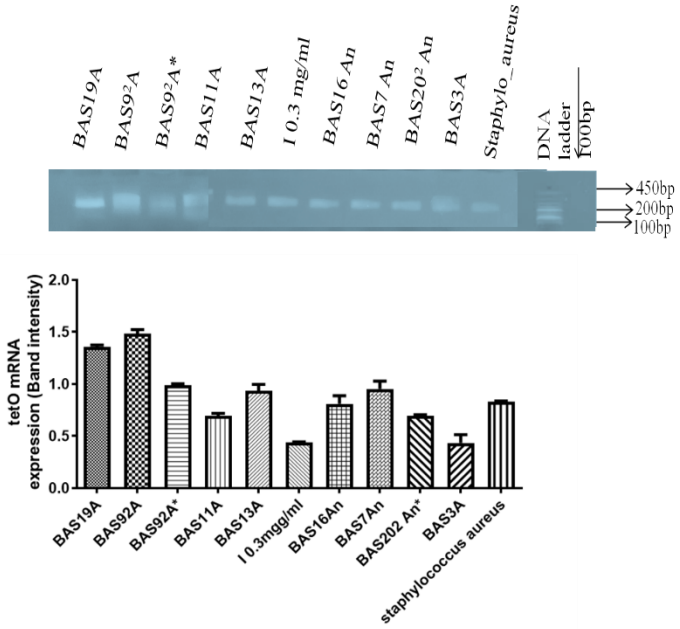

Figure 3: Gel image of *tet*(O) gene expression and its band intensity for 10 representative samples of GBS

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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