Original Research Article

Antibacterial Assessment of Crude and Fractionated Extracts of *Vernonia amgydalina* Leaf Against Multiple Antibiotic Resistant Bacteria of Wound Infection.

Abstract

This study was conducted to determine the antibacterial effect of crude and fractionated extracts of leaves of *Vernonia amgydalina* against multiple antibiotic resistant bacteria isolate from wounds. The bacteria isolates obtained are: *Staphylococcus aureus, Escherichia coli*, *Pseudomonas aeuroginosa*, *Klebsiella pneumoniae*, *Proteus mirabilis*, and *Streptococcus pyogene;* they were confirmed standard microbiological techniques. Extracts were prepared from the leaf of the plant using ethanol, chloroform, cold water and hot water. The antibacterial activities of the crude extracts were assayed using the agar well diffusion methods. Multiple antibiotic resistant of the isolates was confirmed in the six (6) isolates. The phytochemical screening revealed the presence of alkaloids, phenols and saponins. All the extract showed varying degrees of activities depending on the extract concentration and the extract solvent. Generally, ethanol rated best as the extraction solvent which was able to inhibit all isolates except *K. pneumoniae* and *P. mirabilis, a*nd inhibits *S. aureus* at 50mg/ml with zones 6.997 ± 0.003**.** The antibacterial efficacy of the extract increases with a corresponding increase in the extract concentration. The MBC values ranged from 50 - 400 mg/mL for all the extraction solvent, and this is very important for people who depend on the plant for their health care needs. Fra 1, 6, and 10 of the column fractions were able to inhibit with 8.997 $\pm$ 0.008mm, 8.993 $\pm$ 0.012mm and 9.007 $\pm$ 0.000 mm respectively. The functional groups are Alkene, alkane group, alkyl halides, N-H bend of amides and Alcohol. The leaf of *Vernonia amgydalina* can be used as a raw material for the production of new antibiotic to control multiple resistant bacteria.

Keywords: Wound, Bacteria, *Vernonia amgydalina*, Antibacterial, Multiple Antibiotic Resistance.

1.0. INTRODUCTION

Wound infection is a major health problem resulting from colonization of wounds by pathogenic organisms [1]. Bacterial infections caused by multiple antibiotic resistant (MAR) bacteria are a growing threat worldwide [2]. Plants have the major advantage of still being the most effective and cheaper alternative source of drugs [3].*Vernonia amygdalina*, a member of the *Asteraceae* family, is a small ever green shrub that grows in tropical Africa. It is a shrub of 1 - 3 m in height with petiole leaf of about 6 mm in diameter and elliptical in shape [4]. The plant is mostly found in West Africa where the most used part is its leaves (called bitter leaf). The leaves are dark green in colour with a characteristic odour and when chewed has a bitter taste but delicious in meals due to its pleasant nostalgic bitterness when it interacts with protenous ingredients (such as fresh or dry fish) in the soup. Local names by which the plant is called in Nigeria include: Kiriologbo (in Ijaw); Onugbu (in Igbo), Ewuro (in Yoruba) and Shiwaka (in Hausa). The leaves and bark in Ethiopian local medicine are used as purgative, against menstrual pain and wound dressing [5, 6]. It is also documented that *V. amygdalina* has been used traditionally in blood clotting and has elicited a significant reduction in blood glucose levels at post-prandial time point. [7,8] reported that *V. amydalina* has hypoglycaemic activity. They observed a dose-dependent reduction in fasting blood sugar level in alloxan-induced diabetic rats after treatment with different concentrations of the aqueous leaf extracts. The aqueous and ethanol extracts of these plant leaves were tested against *Escherichia coli, Staphylococcus aureus, Bacillus cereus, Shigella dysentriae and Salmonella typhimurium* in which the plants showed antibacterial activity on the entire test isolates [9-11].

2.0 MATERIALS AND METHOD

2.1. Isolation and identification

Pure cultures of wound isolates were obtained from the State specialist Hospital, Akure, Ondo State. The bacteria isolates obtained are *Staphylococcus aureus, Escherichia coli*, *Pseudomonas aeuroginosa*, *Klebsiella pneumoniae*, *Proteus mirabilis*, and *Streptococcus pyogenes. These isolates were* confirmed using standard techniques. [12].

2.2. Antibiotics susceptibility profile

Antibiotic susceptibility testing was performed using the Kirby Bauer disk diffusion method [13] and interpretation according to the Clinical and Laboratory Standards Institute (CLSI) guidelines [14].

2.3. Collection of plant

The fresh leaves of *Vernonia amygdalina* were collected from Elesare, Ondo state and authenticated at the Crop Soil and Pest Management department, Federal University of Technology Akure, Ondo State. The collected plants were washed in distilled water, dried and ground. The crude extracts were obtained by soaking 100 grams of dried plant leaves and stem powder in 1000mL of Hot water, Cold water, Ethanol and Chloroform separately for 72 hrs and sieved with a muslin cloth. Extract were further concentrated by using a rotary vacuum evaporator at 45-50 °C and stored.

2.3.1 Phytochemical analysis of plants

Qualitative and quantitative phytochemical analyses were carried out on the extracts using standard chemical methods as described by [15, 16].

2.3.2. Reconstitution of Extracts

The test solution of each extract was prepared by dissolving 50mg-400mg of the plant extracts separately in 1ml of already prepared Tween 20(20%) to get a concentrations of each extract 50 mg/mL, 100mg/mL, 200mg/mL, 300mg/mL, and 400mg/mL [17].

2.4. Standardization of innoculum

0.5 Mcfarland Standard was used which is equivalent to approximate Cell counts density of $1.5x10^8$. This was obtained by inoculating the test organisms into liquid broth and incubated for 24hrs at 37^0C. 0.5ML of the 24hr. Old broth culture was then inoculated into a new broth and it is then incubated for about 3-5 hrs. This was then streaked with sterile cotton swab on the molten media [18].

2.5. Determination of antibacterial activities of extracts

2.5.1. Antibacterial assay

The pour plate method was adopted. One (1) mL of standardized Inoculum (matching 0.5 McFarland turbidity standards) of various test isolates in nutrient broth containing the test organism was introduced into a sterile Petri dish and an already prepared sterile medium was allowed to cool, and then poured into the plates containing the isolates. The plates were agitated and allowed to solidify. Wells

were dug in the plate using a cork bore of size 80mm. The extract was reconstituted with Tween 20, and varying concentration of the extract was prepared which are: 50mg/mL -400mg/mL and filter sterilized with membrane filter. Tween 20 was incorporated in Mueller Hinton agar well which served and solvent controls respectively zones of inhibition were determined after 24h incubation at 37°C, and the results were measured and recorded [14].

2.5.2. Minimum inhibitory concentration and minimum bactericidal concentration

The determination of Minimum inhibitory concentration of the extract was carried out using broth dilution method. Varying concentrations of each extract (50-400 mg mL^{-1}) were prepared by using serial dilution method. Five (5) microliters of standardized Inoculum (matching 0.5 McFarland turbidity standards) of various test isolates in nutrient broth was put into each test tube. 0.5 mL of each extracts, separately at each concentration were added into the test tubes and thoroughly mixed. The tubes were then incubated at 37^{o}C for 24hours and observed for growth of bacteria in the form of turbidity using spectrophotometric end points methods. The MBC was determined by taking a loopful from each tube that showed no growth during MIC determination and streaked onto Mueller Hilton agar plates and incubated at 37°C for 24 hours. The results were measured and recorded [14].

2.6. Fractionation of plant extract

The Ethanol crude extract of *Vernonia amygdalina* was chromatographed on silica gel (60-120 mesh size) matrix packed into a glass column and eluted successively with ethyl acetate 100%, Ethyl acetate : methanol (3:1), methanol : Ethyl acetate (3:1) and and 100% methanol. The sample was mixed with a little gel to form powder, and was then carefully poured on top of the packed silica gel in the column. It was then covered with glass wool to avoid spattering of the eluant on the extract which may affect the separation process. The eluted fractions were collected in 100mL conical flask [19, 20].

2.7. Thin layer chromatography

Collected fractions were examined by thin layer chromatography (TLC). The method of Harborne [21] was adopted. The different fractions were spotted on a pre-coated (silica gel 60 F254) aluminium plates and eluted with ethyl acetate and chloroform (30: 70) in a small TLC tank. The distance moved by the sample and the distance moved by the solvent were recorded. The ratio of the distance moved by the

sample and the solvent gave the Resolution front (Rf). The fractions with similar Rf values were pooled together as similar compounds.

2.8. Antimicrobial assay of the fractions

The pour plate method was adopted. One (1) ml of standardized inocula (matching 0.5 McFarland turbidity standards) of various test isolates in nutrient broth containing the test organism will be introduced into a sterile Petri dish and an already prepared sterile medium will be cooled and poured on it. The plate was agitated and allowed to solidify. Wells was be dug in the plate using a cork bore of size 80mm. the extract was reconstituted with Tween 20, and filter sterilized with membrane filter. Tween 20 was incorporated in Mueller Hinton agar well which served and solvent controls respectively zones of inhibition were determined after 24h incubation at 37°C and the results were measured and recorded [14].

2.9. Fourier transmission infrared spectrometry

Characterization was carried out after fractionation with column chromatography. The fraction so obtained were subjected to Infrared spectroscopy, Aliquot of 5ml sample was measured according to standard procedure. Each KBr were scanned at 4mm/s at a resolution of 2cm over a wave number region of 400-4500cm-1. The characteristic peaks were recorded using Perkin Elmer Spectrophotometer (model 180).

2.10. Statistical analysis

All the experiments were carried out in triplicate and data obtained from the study were subjected to one-way analysis of variance. Treatment means were compared using Duncan's New Multiple Range Test (DNMRT) at 5% level of significance and questionnaires were analysed using chi square with the aid of SPSS version 20.

3.0. RESULTS

Table 1a: Antimicrobial susceptibility patterns of Gram negative bacteria.

Isolates	CN	PEF	OFL	S	SXT	CH	SP	CPX	AM	AU
	Zones of Inhibition (mm)									
E.coli	0.000±0.000^{a}	15.007±0.012^{d}	12.997±0.009^{b}	0.000±0.000^{a}	0.000±0.000^{a}	0.000±0.000^{a}	0.000±0.000^{a}	0.000±0.000^{a}	13.003±0.008^{c}	0.000±0.000^{a}

P. mirabilis	0.000±0.000[a]	0.000±0.000[a]	0.000±0.000[a]	0.000±0.000[a]	0.000±0.000[a]	0.000±0.000[a]	0.000±0.000[a]	0.000±0.000[a]	0.000±0.000[a]	0.000±0.000[a]
K. pneumoniae	0.000±0.000[a]	0.000±0.000[a]	0.000±0.000[a]	0.000±0.000[a]	0.000±0.000[a]	0.000±0.000[a]	0.000±0.000[a]	0.000±0.000[a]	12.003±0.008[b]	0.000±0.000[a]
P. aeruginosa	0.000±0.000[a]	0.000±0.000[a]	0.000±0.000[a]	0.000±0.000[a]	0.000±0.000[a]	0.000±0.000[a]	0.000±0.000[a]	0.000±0.000[a]	0.000±0.000[a]	0.000±0.000[a]

KEY: CN- Gentamycin 10 μg, S- Streptomycin 30 μg, PEF- Pefloxacin 10 μg, OFL- Tarivid 10 μg, SXT-Septrin 30μg, CH- Chloramphenicol 30μg, SP- Sparfloxacin 10 μg, CPX- Ciprofloxacin 10 μg, AM-Amoxacillin 30 μg, AU-Augumentin 30 μg.
Data are represented as mean± SE (standard error). Each value is a mean of three (3) replicates
Values with the same superscript letters along the same column are not significantly different ($p \leq 0.05$).

Table 1b: Antimicrobial susceptibility patterns of Gram positive bacteria.

Isolates	CN	PEF	S	SXT	CPX	AM	APX	E	Z	R
	Zones of inhibition(mm)									
S. pyogenes	0.000±0.000[a]	15.007±0.012[c]	0.000±0.000[a]	0.000±0.000[a]	0.000±0.000[a]	0.000±0.000[a]	10.997±0.003[b]	0.000±0.000[a]	0.000±0.000[a]	0.000±0.000[a]
S. aureus	0.000±0.000[a]	6.007±0.176[b]	16.003±0.014[e]	16.997±0.003[f]	10.993±0.012[c]	0.000±0.000[a]	0.000±0.000[a]	11.997±0.003[d]	0.000±0.000[a]	0.000±0.000[a]

KEY: CN- Gentamycin 10 μg, S- Streptomycin 30 μg, PEF- Pefloxacin 10 μg, SXT-Septrin 30μg, CPX-Ciprofloxacin 10 μg, AM- Amoxacillin 30 μg, APX-Ampiclox 30μg, E- Erythromycin 10 μg, Z-Zinnclof 20 μg, R-Rocephin 25\ μg.

Data are represented as mean± SE (standard error). Each value is a mean of three (3) replicate . Values with the same superscript letters along the same column are not significantly different ($p \leq 0.05$).

Table 1a and 1b revealed the antibiotic sensitivity patterns of the isolates. *P. mirabilis,*and *P. aeruginosa* were resistant to all the antibiotics used: *E.coli*, *K. pneumonie*, and *S.pyogenes* were sensitive to only one antibiotics:, *A. hydrophila* was sensitive to seven of the 10 antibiotics used.

Table2: Phytochemical analysis of *Vernonia amygdalina*

Phytochemical	Ethanol	Chloroform	Cold Water	Hot Water
Flavonoid	+	-	-	+
Saponin	+	+	+	+
Tannins	+	+	+	+
Alkaloid	-	-	-	-
Phenol	-	+	-	-

KEY: + Present - Negative

The qualitative phytochemical properties of the extracts are shown in Table 2. It reveals the presence of Tannins and saponins in all the different extracts of the plant, flavonoid is present in the ethanolic and hot water extract and absent in chloroform and cold water extract.

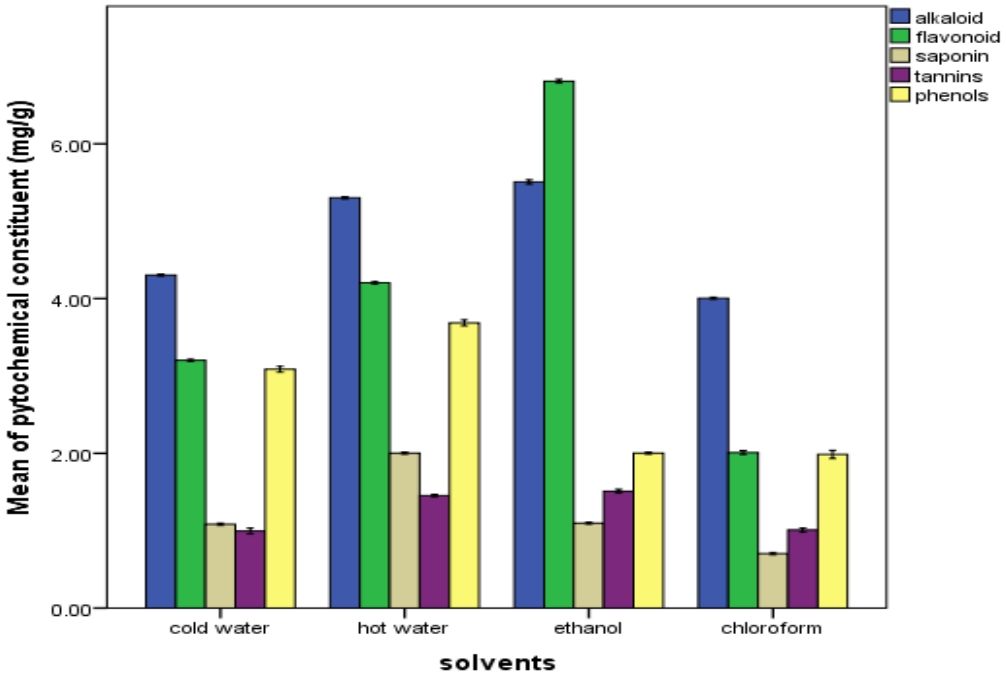

Figure 1: Quantitative phytochemical analysis of *Vernonia amygdalina* extracts

Alkaloid is higher in ethanol extract with 5.507 ± 0.007 and least in cold extract with 4.003 ± 0.003. Saponin is higher in hot water extract with 2.003 ± 0.003 and least in chloroform extract with 0.703 ± 0.003. Flavonoid is highest in ethanol with 6.810 ± 0.006 and least in chloroform extract with 2.010 ± 0.007. Phenol is higher in hot water extract with 3.780 ± 0.100 and least in chloroform extract with 1.987

± 0.120. Tannin is higher in ethanolic extract with 1.510 ± 0.006 and least in cold water extract with 0.997 ± 0.008.

Table 3 shows the effect of antimicrobial on the multiple antibiotic resistance bacteria at different concentration. At 50mg/mL the extract was only able to inhibit *E.coli* with 5.990±0.010. At 200mg/mL the zones ranges from 0.000±0.000 - 8.000±0.000, at 200mg/mL it ranges from 0.000±0.000- 9.997±0.015, at 300mg/mL it ranges from 0.000±0.000 - 12.007±0.007 and at 400mg/mL it has its highest zone on *E.coli* with 13.003±0.009 and the least on *P. mirabilis* with 2.987±0.013. At 50mg/mL the hot water extract was only able to inhibit *E.coli* with 5.990±0.010. At 200mg/mL the zones ranges from 0.000±0.000 - 8.000±0.000, at 200mg/mL it ranges from 0.000±0.000- 9.997±0.015, at 300mg/mL it ranges from 0.000±0.000 - 12.007±0.007 and at 400mg/mL it has its highest zone on *E.coli* with 13.003±0.009 and the least on *P. mirabilis* with 2.987±0.013. The effect of ethanol extract of *V. amygdalina* leaf on the bacterial isolates. At 50mg/Ml the chloroform extract was only able to inhibit *E.coli* with 5.990±0.010, at 200mg/mL the zones ranges from 0.000±0.000 - 8.000±0.000, at 200mg/mL it ranges from 0.000±0.000- 9.997±0.015, at 300mg/mL it ranges from 0.000±0.000 - 12.007±0.007 and at 400mg/mL it has its highest zone on *E.coli* with 13.003±0.009 and the least on *P. mirabilis* with 2.987±0.013. At 50mg/mL of chloroform extract was only able to inhibit *E.coli* with 5.990±0.010, at 200mg/mL the zones ranges from 0.000±0.000 - 8.000±0.000, at 200mg/mL it ranges from 0.000±0.000- 9.997±0.015, at 300mg/mL it ranges from 0.000±0.000 - 12.007±0.007 and at 400mg/mL it has its highest zone on *E.coli* with 13.003±0.009 and the least on *P. mirabilis* with 2.987±0.013.

Table 3: Antibacterial effect of the extracts on the bacterial isolates

Isolates		50mg/mL	100mg/mL	200mg/mL	300mg/mL	400mg/mL	Tween 20
				Zones of Inhibition (mm)			
E. coli	**Hot water**	**5.990±0.010**	**7.003±0.003**	**9.997±0.015**	**12.007±0.007**	**13.003±0.009**	**0.000±0.000**
	Cold water	**0.000±0.000**	**0.000±0.000**	**10.000±0.006**	**10.507±0.012**	**11.003±0.009**	**0.000±0.000**
	Ethanol	**2.000±0.006**	**9.013±0.019**	**9.990±0.015**	**11.000±0.06**	**11.993±0.012**	**0.000±0.000**
	chloroform	**0.000±0.006**	**0.000±0.000**	**0.000±0.000**	**0.000±0.000**	**1.000±0.000**	**0.000±0.000**
S. pyogenes	**Hot water**	**0.000±0.000**	**6.010±0.015**	**7.000±0.000**	**7.500±0.006**	**7.990±0.015**	**0.000±0.000**
	Cold water	**0.000±0.000**	**0.000±0.000**	**0.000±0.000**	**0.000±0.000**	**2.004±0.008**	**0.000±0.000**
	Ethanol	**2.520±0.015**	**8.003±0.003**	**8.010±0.015**	**10.003±0.014**	**15.017±0.017**	**0.000±0.000**
	chloroform	**0.997±0.003**	**5.000±0.000**	**7.007±0.007**	**7.503±0.009**	**8.000±0.000**	**0.000±0.000**
P. mirabilis	**Hot water**	**0.000±0.000**	**0.000±0.000**	**0.000±0.000**	**0.000±0.000**	**2.987±0.013**	**0.000±0.000**

	Cold water	0.000±0.000	0.000±0.000	0.000±0.000	0.000±0.000	2.003±0.009	0.000±0.000
	Ethanol	0.000±0.000	0.000±0.000	7.167±0.015	7.997±0.008	10.003±0.014	0.000±0.000
	chloroform	2.010±0.015	4.990±0.015	10.000±0.000	10.000±0.000	11.000±0.000	0.000±0.000
K. pneumoniae	Hot water	0.000±0.000	8.000±0.000	9.017±0.016	9.000±0.000	10.007±0.018	0.000±0.000
	Cold water	0.000±0.000	0.000±0.000	10.003±0.009	11.507±0.012	13.007±0.012	0.000±0.000
	Ethanol	0.000±0.000	0.000±0.000	6.997±0.008	9.003±0.008	10.007±0.012	0.000±0.000
	chloroform	4.000±0.011	8.003±0.003	9.990±0.010	10.000±0.000	10.017±0.016	0.000±0.000
S. aureus	Hot water	0.000±0.000	0.000±0.000	8.00±0.000	9.9003±0.003	10.990±0.015	0.000±0.000
	Cold water	0.000±0.000	6.003±0.015	10.007±0.012	10.997±0.009	12.013±0.008	0.000±0.000
	Ethanol	6.997±0.003	11.020±0.010	15.010±0.012	16.010±0.006	19.010±0.015	0.000±0.000
	chloroform	2.997±0.015	7.000±0.014	7.003±0.015	9.010±0.000	11.997±0.003	0.000±0.000
P. aeruginosa	Hot water	0.000±0.000	7.017±0.000	9.983±0.017	10.003±0.003	12.000±0.000	0.000±0.000
	Cold water	0.000±0.000	0.000±0.000	0.000±0.000	0.000±0.000	1.013±0.009	0.000±0.000
	Ethanol	0.997±0.003	3.987±0.013	5.993±0.007	6.493±0.012	8.013±0.009	0.000±0.000
	chloroform	0.000±0.000	1.000±0.000	4.017±0.012	6.000±0.000	8.010±0.010	0.000±0.000

Table 4: Minimum inhibitory concentration (MIC) and Minimum bactericidal concentration (MBC) of *V.amygdalina* leaf extracts on isolates

Isolates	Hot water		Cold water		Ethanol		Chloroform	
	MIC (mg/mL)	MBC (mg/mL)	MIC (mg/mL)	MBC (mg/mL)	MIC (mg/mL)	MBC (mg/mL)	MIC (mg/mL)	MBC (mg/mL)
E. coli	50	100	200	400	50	100	400	400
S. pyogenes	100	200	400	400	50	100	50	100
P. mirabilis	400	400	400	400	200	400	50	100
K. pneumoniae	100	200	200	400	200	400	50	100
S. aureus	200	400	100	200	50	100	50	100
P. aeruginosa	100	200	400	400	50	100	100	200

Table 4 presents the MIC, MBC of the extract. For hot water extract, the MIC on the bacterial isolates ranges from 50mg/mL – 400mg/mL and MBC ranges from 100mg/mL – 400mg/mL. for the cold water extract, the MIC on the bacterial isolates ranges from 100mg/mL – 400mg/mL, while the MBC ranges from 200mg/mL – 400mg/mL. For the ethanolic extract, the MIC on the bacterial isolates ranges from 50mg/mL – 200mg/mL, while the MBC ranges from 100mg/mL – 400mg/mL. for the chloroform

extract of *V. amygdalina*, the MIC on the bacterial isolates ranges from 50- 400mg/mL, while the MBC ranges from 100mg/mL - 400mg/mL.

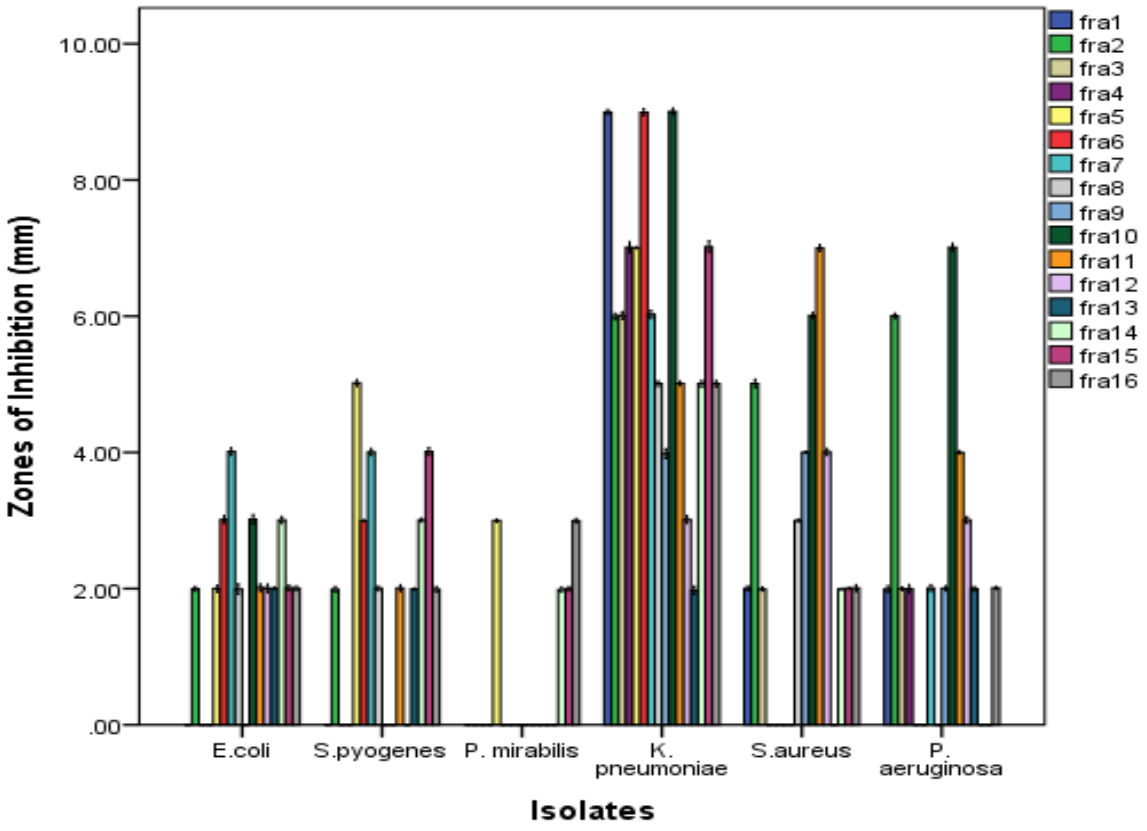

Figure 2: Antibacterial effect of column fractions of *V. amygdalina* on the bacterial isolates

Key: Fra- Fractions of column

Fraction 1, 6 and 10 has the highest inhibitory effect on the isolates with 8.997 ± 0.008mm, 8.993 ± 0.012mm and 9.007 ± 0.000 mm respectively. Generally, all the fractions have inhibitory effect on *Klebisella pneumoniae.*

Figure 3- 5 shows the different appearance with the corresponding wave length of Fraction EAV1, EAV6 and EAV10. This active compounds common to the three are N - H group and the compound is Amine, C=O group, the compound is aldehyde, S= O group the compound is sulfonamide, and C=C group which is the compound is Alkene

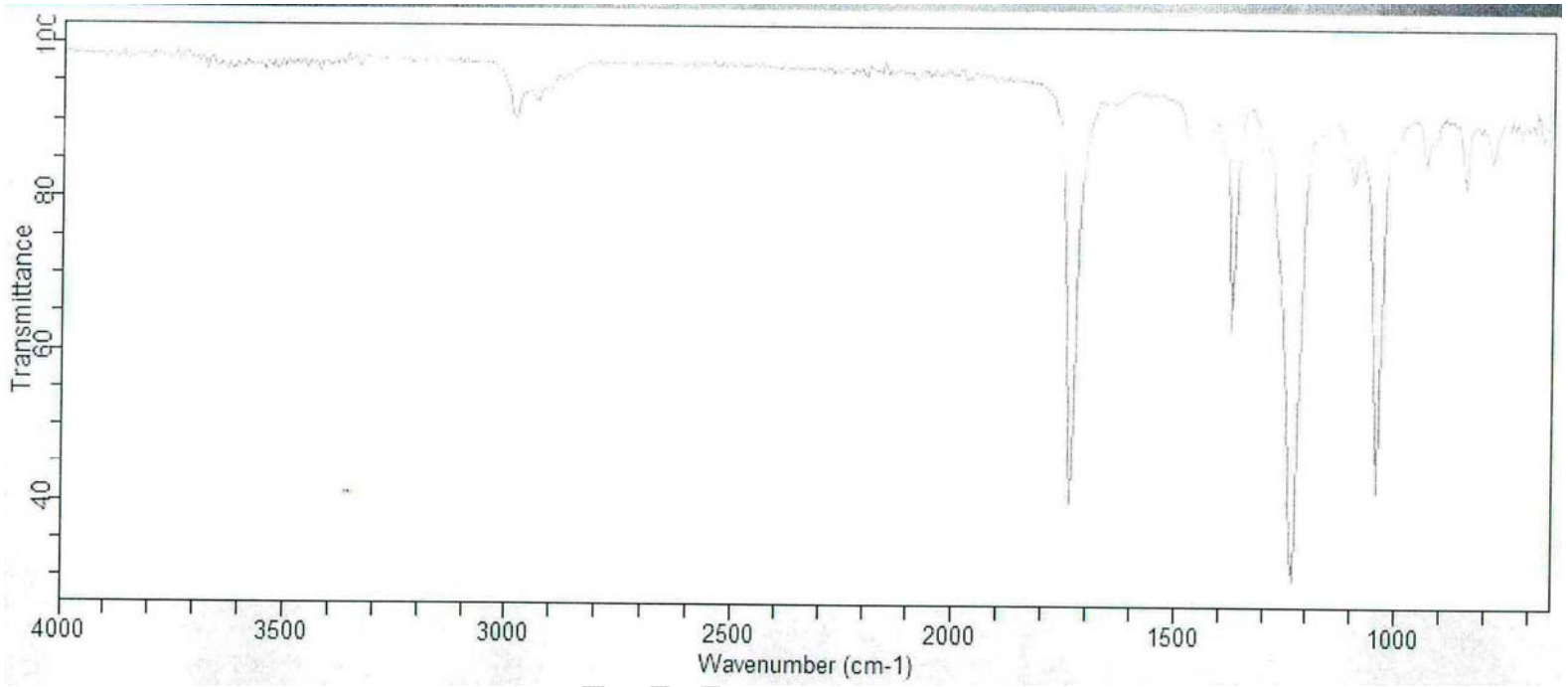

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Figure 3: The Fourier Transmission Infrared Spectrometry of FRA EAV1

1233 667

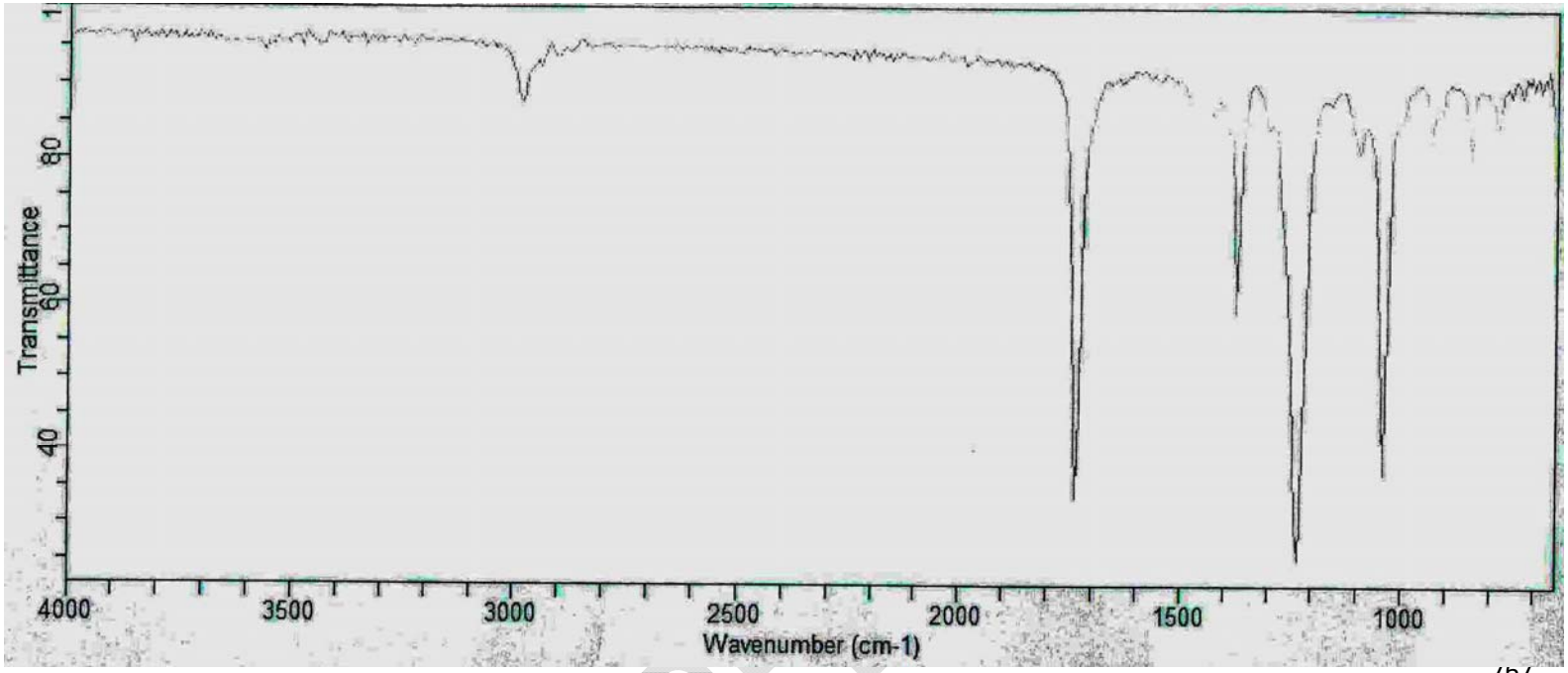

Figure 4: The Fourier Transmission Infrared Spectrometry of FRA EAV10

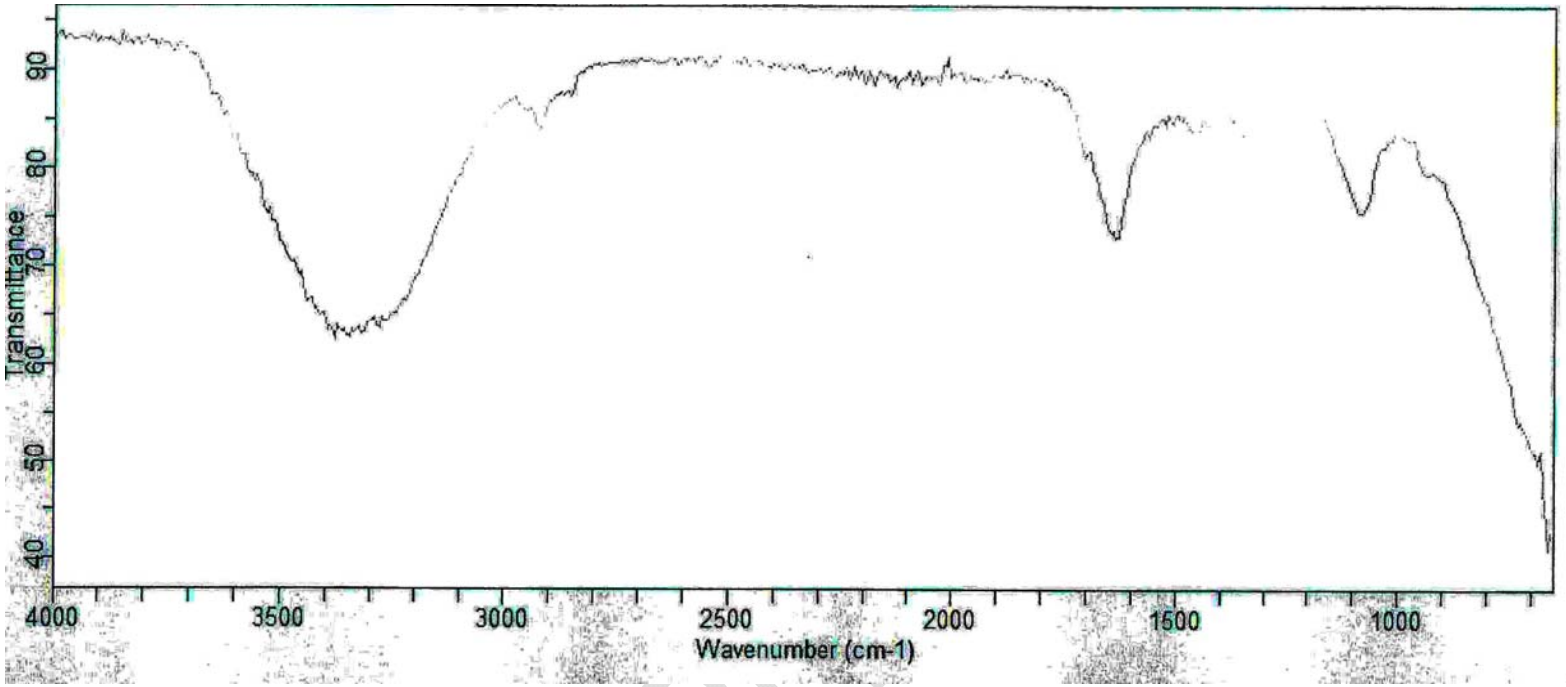

Figure 5: The Fourier Transmission infrared Spectrometry of FRA EAV6

DISCUSSION

The bacteria used in this study*; Staphylococcus aereus*, *Escherichia coli*, *Pseudomonas aeuroginosa*, *Klebsiella pneumoniae*, *Proteus mirabilis*, *Streptococcus pyogenes* are the common bacteria usually associated with wound in agreement with [22-23]. All isolates used exhibited multiple antibiotic resistances. Isolates were resistant to five (5) or more antibiotics. *P. mirabilis, P. aeruginosa* were resistant to all the antibiotics of the gram positive antibiotic disc. The high rate of resistance towards these antibiotics may be due to their overuse because they are readily available over the counter, substitution of dose without finishing the initial dose [22]. The extraction solvent used for this study include; Ethanol, Chloroform, Hot water and Cold water. Ethanol yielded a higher end concentration compared to other extraction solvent used. Ethanol has the ability to extract bioactive compounds like tannis, saponnins, flavonoid, alkaloid, phenol and steroid which have higher concentrations in *V. amygdalina* as reported by [24]. Ethanolic extracts of *vernonia amygdalina* at varying concentration was able to inhibit all the test bacteria, especially at higher concentration. This is similar to the report of [9, 11, and 24] who all worked on the effect of *V. amygdalina* on clinical isolates. The *V. amygdalina* leaves hot water extract has the least inhibitory effect on the test isolates compared with the ethanoic leaf extract , followed by the chloroform extract and the cold water extract with the least. [25, 26] observed that the presence of secondary metabolites such as, tannins, flavonoids, phenol, anthraquinone, chalcones, cardenolides, glycosides, quinines, terpenoids, saponins and fixed oil was observed in the various plant preparations of *V. amygdalina,* also that the secondary metabolites could be responsible for the biological activity of the plants. *Proteus mirabilis* which was resistant to all the antibiotics was sensitive to higher concentration of leaf chloroform and ethanolic extract *V. amygdalina*. *Pseudomonas aeruginosa* which showed resistance to all the antibiotics was sensitive to all the extracts, except for cold water extract of *V. amygdalina*. This is similar to what [9] reported of *V.amygdalina* that at high concentrations and observable time limit, there could be bactericidal effect of the extract on organism. He also reported that ethanolic extracts of *V. amygdalina* has higher antibacterial effect compared to the cold water extract. The minimum inhibitory concentration for all the extracts ranges from 50mg/ml to 400mg/ml. the minimum bactericidal concentration for all the extract varies for each solvent and bacterial isolate for *V.amygdalina*. [27] reported that the minimum inhibitory concentration of the *V. amygdlaina* extracts on *S. aureus* from

wound samples vary between 12.5 and 100 mg/ml; while the minimum bactericidal concentration of extracts vary between 50 and 200 mg/ml. The fractions of the ethanolic extract of *V.amygldalina* have varying inhibitory effect on the bacterial isolates. The sixteen (16) fractions have inhibitory effect on *Klebsiella pneumoniae*. From the sensitivity patterns of the bacterial isolates to the fractions of *V. amygdalina*, it is observed that there is a synergistic relationship between the extract as a whole. [28] reported that the two sesquiterpene lactones (vernolide and vernodalol) isolated from *V. amygdalina* exhibited appreciable antioxidant activities as manifested through their reducing capacity and free radical (DPPH) scavenging properties. The ethanol extract, however, had the highest free radical scavenging activity, therefore suggesting a possible synergistic effect of these compounds and other constituents. [29] also reported that *V.amygdalina* have synergistic effect.

The bioactive functional group in the fractions of the ethanolic extract of *V.amygdalina* is the C=C group. The compound is Alkene; it is present in the three (3) fractions that have higher antimicrobial properties against the bacterial isolates among the sixteen (16) fractions. This is similar to what [30] reported of *V. amygdalina,* that the Alkene group-Vernodalinol, is the active fractions of *Vernonia amygdalina* ethanol extract. Vernodalinol was also reported to have antibacterial activities [30]. S=O group with sulfonamide is common to the fraction 1 and 10. Fraction 1 and 10 has similar functional group and compounds. The presence of a phenol ring with an OH stretch, alkane group, alkyl halides, N-H bend of amides, a double bond stretch of aldehyde and a benzene ring which suggest the presence of the possible compounds, flavonoids, Saponins, Phenolic compound and alkaloid [31]. Considering the extract from *Vernonia amygdalina* studies suggest the present of phenolic and saponin compounds or with benzene related compound that has functional group(s) of OH, alkanes side chain, alcohol, carbonyl and aldehyde [32].

CONCLUSION

This study has shown ethanol as the best extraction solvent for *V. amygdlaina.* It also highlighted Alkene group as bioactive component in the antibacterial effect *V.amygdalina* extracts. There is a higher possibility of using extracts of *Annona muricata* and *Vernonia amygdalina* as an alternative control or reduce multiple antibiotic resistance in wound infection.

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