Microbes and Infectious Diseases 2024; 5(3): 1228-1239

Microbes and Infectious Diseases

Journal homepage: https://mid.journals.ekb.eg/

Original article

Antibacterial activities of *Vernonia amygdalina* (Del.) stem bark extracts on multiple antibiotic-resistant bacteria isolated from wound samples

Anthony Kayode Onifade, Damilola Deborah Akinyemi *, Ayodele Oluyemisi Ogundare

Department of Microbiology, Federal University of Technology, PMB 704, Akure, Ondo State, Nigeria.

ARTICLE INFO

Article history:
Received 22 June 2023
Received in revised form 7 July 2023
Accepted 12 July 2023

Keywords:
Wound
Antimicrobial resistance
Bacteria
Vernonia amygdalina
Inhibition zone

ABSTRACT

Introduction: *Vernonia amygdalina (V. amygdalina)* (Del.) has been reported to have medicinal values, and the phytochemical constituents which is present in different parts of the plant have been found to be responsible for the antimicrobial efficacy of the plant. This study focuses on the antibacterial activity of the stem bark extracts of *V. amygdalina* against multiple antibiotic-resistant (MAR) bacteria isolated from wound samples and characterize the bioactive compounds present in the plant. **Methods:** Bacteria were isolated from wound samples using standard microbiological techniques and disc diffusion method was used to determine the sensitivity patterns of the isolated bacteria to conventional antibiotics and to the various extracts of *V. amygdalina* stem bark. Ciprofloxacin was used as control during the antibacterial assay. GC-MS analysis was carried out to identify the bioactive compounds in the stem bark extracts of the plant. **Results:** *Pseudomonas aeruginosa* (36%), *Staphylococcus aureus* (29%), *Staphylococcus epidermidis* (14%), *Bacillus subtilis* (6%), *Escherichia coli* (6%), *Klebsiella pneumoniae* (5%), and *Proteus mirabilis* (4%) were the bacteria isolated from the wound swabs sampled in this study. The ethyl acetate extract of *V. amygdalina* (300 mg/ml) inhibited the growth of these organisms with the greatest effect on *S. aureus* with inhibition zone of 23.17±0.60 mm. The GC-MS analysis of the plant extracts revealed the presence of bioactive compounds such as coumarin and oleic acid, among others. **Conclusion:** The findings from this study have further established that *V. amygdalina* is a promising candidate and effective alternative treatment means for MAR bacteria that are associated with wound infections.

Introduction

Bacterial infection of the wound has been known to be among the leading causes of morbidity and mortality throughout the world [1, 2]. Wound infection poses a global health challenge leading to delay in wound healing and surgical complications like wound breakdown [3]. Wound infection has been identified as the most common nosocomial infection, especially in patients undergoing surgery resulting in a prolonged hospital stay, increased trauma care, treatment costs and more demanding

DOI: 10.21608/MID.2023.219473.1550
* *Corresponding author:* Damilola Deborah Akinyemi
E-mail address: akinyemidd@futa.edu.ng

general wound management practices [3]. *Escherichia coli*, species of *Staphylococcus*, *Pseudomonas*, *Klebsiella*, *Proteus* and anaerobes like *Clostridium* and *Bacteroides* species are frequently found in wound infections.

The majority of plants that were used in ethnomedicine have been studied because of their promising effects against various pathogenic microorganisms [4]. The use of natural products in combination with antibiotics to enhance treatment efficacy is a new strategy developed to overcome the problem of antibiotic resistance [5]. Some of the medicinal plants include *Vernonia amygdalina* [6], *Ocimum gratissimum* [7], *Andrographis paniculata* [8], *Polyalthia longifolia* [9], among others.

The bitter leaf, *V. amygdalina* (Delile) which was used in this study has been reported by some researchers to have nutritional and medicinal values [10,11]. The plant is also known to possess certain phytochemicals such as sesquiterpene lactones, anthraquinones, terpenoids, saponin, tannin, and flavonoids [12,13]. **Ruslim et al.** [14] stated the wound healing potential of *V. amygdalina* indicating that the extract of the plant may hasten wound healing. Extracts of *V. amygdalina* possess antioxidant properties that can hasten wound healing and also has anti-inflammatory and antibacterial effects [15,16]. Therefore, the current study was designed to evaluate the antibacterial activity of the stem bark extracts of *V. amygdalina* on multiple antibiotic resistant bacteria (MARB) isolated from wound samples and characterize the bioactive compounds present in the plant extracts.

Materials and methods

Ethical consideration

Ethical approval was obtained from the Health Research Committee, Ministry of Health, Akure, Nigeria. Protocol number: OSHREC 20/8/2021/366. Date of approval: 20/8/2021.

Isolation and identification of bacteria

A total of 93 wound swab samples were obtained from patients attending the University of Medical Sciences Teaching Hospital (UNIMEDTH), Akure, Nigeria after administering questionnaire. Bacteria was isolated and identified using standard microbiological procedures according to **Cheesbrough** [17] and **Ijabani et al.** [18].

Antibiotic sensitivity testing

The inoculum was standardized using 0.5 McFarland's standard as described by **Isunu et al.** [19]. Antibiotic susceptibility test was performed using Kirby-Bauer disc diffusion method described by **Cheesbrough** [17]. The diameter of zones of inhibition was measured and interpreted using standard interpretative charts as recommended by the Clinical and Laboratory Standards Institute [20].

Collection of plant materials

The stem bark of *V. amygdalina* were collected from a farm in Akure, Nigeria. The plant samples were air-dried before being used for the research.

Preparation of plant extracts

The air-dried stem bark samples of *V. amygdalina* were powdered using an electric blender. The powdered plant materials were extracted using methanol, ethyl acetate, hot water, and cold water. A 100g portion of the powdered sample was dissolved in 1000ml of each of the extraction solvents in a conical flask. The mixture was kept in a shaker for 72h after which the extract was drained out using muslin cloth and filtered with Whatman No 1 filter paper. The extracts were concentrated to dryness using a rotary evaporator. The extracts were preserved in air-tight containers at 4ºC for further use [21].

Phytochemical analysis

The presence and amounts of phytochemicals in the different solvent extracts of *V. amygdalina* stem bark were determined using standard methods described by **Trease et al.** [22], **Sofowora** [23] and **Harborne** [24].

Antibacterial activity of *V. amygdalina*

The assay for the antibacterial activity of *V. amygdalina* extracts was carried out using method described by **Kone et al.** [25]. The reconstitution of the extracts was done to give various concentration intended for use in this study. Sterile perforated filter papers were then impregnated with the reconstituted extracts and placed accordingly on Mueller-Hinton agar plates that have been streaked with the test organisms. Ciprofloxacin 2mg/ml and DMSO was used as positive and negative control respectively. The plates were then incubated for 18 hours at 37 ℃ after which the diameter of zones of inhibition was measured in mm.

Determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)

The MIC assay was carried out using broth dilution method with peptone water broth as described by **Kone et al.** [25]. Test tubes containing different concentrations of the extracts of *V. amygdalina*,

ranging from 6.25 mg/ml to 200 mg/ml were inoculated with the standardized bacteria and incubated for 18 – 24 hours. The lowest concentration of *V. amygdalina* extracts that shows no visible turbidity or growth of the bacterial isolates was recorded as the MIC.

The MBC assay of the extracts was determined using the method described by **Kone et al.** [25]. The test tubes from the MIC test that did not show visible growth were aseptically inoculated on different sterile Muller-Hinton agar plates and incubated at 37 °C for 24 hours. The MBC was chosen as the lowest extracts concentration that resulted in no visible growth of the bacterial isolates on the plate.

Characterization of bioactive compounds in *V. amygdalina* using GC/MS

The method of **Gopu et al.** [21] was used for characterization of the bioactive compounds and their percentage abundance using a Varian GC – MS equipment (Varian 4000 mass spectrometer, USA) alongside a mass spectrometer (MS) 3000 equipped with Agilent MS capillary column (30 m length × 0.25 mm diameter × 0.25 µm thickness).

Statistical analysis

Data obtained in this study were subjected to One-Way Analysis of Variance (ANOVA), and differences between means were separated using Duncan's New Multiple Range Test at 5% level of significance using Statistical Package for Social Sciences (SPSS) version 26.0.

Results

Identification of bacterial isolates

The distribution of sample in relation to age and sex is represented in **figure (1)**. **Table 1** shows the biochemical characteristics of the bacteria isolated from wound swabs in this study. These include *Bacillus subtilis, Escherichia coli (E. coli), Klebsiella pneumoniae (K. pneumoniae), Proteus mirabilis (P. mirabilis), Pseudomonas aeruginosa (P. aeruginosa), Staphylococcus aureus (S. aureus),* and *Staphylococcus epidermidis.*

Percentage occurrence of bacteria isolated from wound swab samples

The percentage occurrence of the bacteria isolated from the wound swab samples is presented in **figure (2).** The most frequently occurring bacterium in this study was *Pseudomonas aeruginosa* (36%), followed by *Staphylococcus aureus* (29%) while *Proteus mirabilis* (4%) was the least frequently occurring bacterium among the isolates obtained in this study.

Antibiotic sensitivity pattern of bacterial isolates

The antibiotic sensitivity pattern for Gram-positive and Gram-negative bacteria are shown in **tables (2 and 3)** respectively. The isolates were highly resistant to augmentin and amoxicillin.

Phytochemical constituents of the plant extracts

The qualitative phytochemical analysis revealed that both phlobatannins and anthraquinones are absent in all the extracts. It also confirms the presence of saponin, tannin, flavonoid, terpenoids and cardiac glycosides in all the extracts as presented in **table (4). Table 5** shows the quantitative phytochemical constituents and it reveals saponin as the most abundant phytochemical in all the extracts.

Antibacterial activity of *V. amygdalina*

The antibacterial activities of *V. amygdalina* stem bark various extracts are shown in **tables(6, 7, and 8)** at 100mg/ml, 200mg/ml and 300mg/ml respectively, while ciprofloxacin and DMSO was used as the positive and negative control respectively.

Minimum inhibitory and bactericidal concentration

The MIC and MBC values are given in **table (9).** The MIC values ranged from 12.5 mg/ml to 100mg/ml across all extracts and organisms tested.

Profile of chemical compounds in the ethyl acetate extract of *V. amygdalina*

The GC-MS analysis of the purified ethyl acetate extract of *V. amygdalina* revealed the presence of bioactive compounds such as n-hexadecanoic acid, methyl ester, oleic acid, 9,12-octadecadienoic acid, octadecanoic acid, cyclohexaneethanol, 9-hexadecenoic acid among others. The compounds with their molecular formula and structures are shown in **table (10)** while the GC-MS chromatographic spectra is shown in **figure (3).**

Table 1. Cultural, Morphological and Biochemical Characteristics of Bacteria Isolated from Wound Swabs.

S/N	Gram	Shape	Elev	LAC	SUC	GLU	H_2S	GAS	IN	MOT	CAT	COA	CIT	OXI	FRU	MR	VP	MAL	MAN	GAL	Probable organism
1	+	Cocci	Raised	+	+	+	-	-	-	-	+	+	+	-	+	+	+	+	+	+	***Staphylococcus aureus***
2	+	Cocci	Raised	+	+	+	+	+	-	-	+	-	+	-	+	-	+	+	-	+	***Staphylococcus epidermidis***
3	-	Rod	Raised	+	+	+	-	+	+	+	+	-	-	-	+	+	-	+	+	+	***Escherichia coli***
4	-	Rod	Raised	+	+	+	-	+	-	-	+	-	+	-	+	+	+	+	+	+	***Klebsiella pneumoniae***
5	-	Rod	Flat	-	-	-	-	+	-	+	+	-	+	+	-	-	-	-	-	-	***Pseudomonas aeruginosa***
6	-	Rod	Raised	-	-	+	+	+	-	+	+	-	+	-		+		-	-		***Proteus mirabilis***
7	+	Rod	Flat	-	+	+	-	-	-	+	+	+	+	-	+	-	+	+	+	+	***Bacillus subtilis***

Key**:** Gram – Grams' staining reaction, Elev- Elevation, LAC – Lactose, SUC – Sucrose, GLU – Glucose, H_2S – Sulphur, IN – Indole production, MOT – Motility, CAT – Catalase, COA – Coagulase, CIT – Citrate, OXI – Oxidase, FRU – Fructose, MR – Methyl red, VP – Voges-Proskauer, MAL – Maltose, MAN – Mannitol, Gal – Galactose, + - Positive, - : Negative

Table 2. Antibiotic Sensitivity Patterns of Gram-Positive Bacterial Isolates from Wound Swab.

	Isolate	Antibiotic zone of inhibition (diameter in mm)									
		PEF	**CN**	**APX**	**Z**	**AM**	**R**	**CPX**	**S**	**SXT**	**E**
1	***S. aureus***	13.67±0.33^{b}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	13.00±0.58^{b}	17.00±0.58^{c}	17.67±0.88^{c}	0.00±0.00^{a}
2	***S. epidermidis***	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	14.00±0.58^{b}	16.67±0.64^{c}	0.00±0.00^{a}	14.33±0.58^{b}
3	***B. subtilis***	25.00±0.58^{b}	20.00±0.30^{a}	25.00±0.33^{b}	22.00±0.58^{ab}	20.00±1.15^{a}	22.32±0.60^{ab}	20.18±1.17^{a}	21.00±0.58^{a}	20.47±0.61^{a}	20.26±0.63^{a}

Key: PEF: Pefloxacin (10µg), CN: Gentamycin (10 µg), APX: Ampiclox (30µg), Z: Zinnacef (20 µg), AM: Amoxicillin (30µg), R: Rocephin (25 µg), CPX: Ciprofloxacin (10µg), S: Streptomycin (30µg), SXT: Septrin (30µg), E: Erythromycin (10µg).
Values are presented as mean±SE of triplicates, values in the same row carrying the same superscript are not significantly different ($p < 0.05$) according to Duncan's New Multiple Range Test

Table 3. Antibiotic Sensitivity Patterns of Gram-Negative Bacterial Isolates from Wound Swab.

Key: SXT: Septrin (30 µg), CH: Chloramphenicol (30 µg), SP: Sparfloxacin (10µg), CPX: Ciprofloxacin (30 µg), AM: Amoxicillin (30 µg), AU: Augmentin (10 µg), CN: Gentamycin (30 µg), PEF: Pefloxacin (30 µg), OFX: Tarivid (10 µg), S: Streptomycin (30 µg)

	Isolate	Antibiotic zone of inhibition (diameter in mm)									
		SXT	**CH**	**SP**	**CPX**	**AM**	**AU**	**CN**	**PEF**	**OFX**	**S**
1	***P. aeruginosa***	7.00±0.58^{b}	17.00±0.58^{c}	17.33±0.33^{c}	23.00±0.58^{d}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	16.00±0.58^{c}	17.00±0.58^{c}	0.00±0.00^{a}
2	***E. coli***	0.00 ±0.00^{a}	10.00 ±0.58^{b}	0.00±0.00^{a}	11.17±0.17^{b}	0.00±0.00^{a}	0.00 ±0.00^{a}	0.00 ±0.00^{a}	0.00±0.00^{a}	11.17±0.44^{b}	11.33±0.33^{b}
3	***K. pneumoniae***	17.00±0.23^{e}	18.00±1.15^{e}	8.33±0.60^{b}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	11.00±0.58^{c}	14.00±0.29^{d}
4	***P. mirabilis***	0.00±0.00^{a}	17.00±0.58^{c}	18.00±1.15^{cd}	20.33±0.60^{de}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	17.30±0.76^{1}	21.67±1.17^{e}	10.17±0.44^{b}

Values are presented as mean±SE of triplicates, values in the same row carrying the same superscript are not significantly different ($p < 0.05$) according to Duncan's

Table 4. Qualitative Phytochemical Constituents of *V. amygdalina* Extracts.

Phytochemical	**Hot water extract**	**Methanol extract**	**Cold water extract**	**Ethyl acetate extract**
Saponin	+	+	+	+
Tannin	+	+	+	+
Phlobatannin	-	-	-	-
Flavonoid	+	+	+	+
Terpenoid	+	+	+	+
Alkaloid	-	+	-	+
Anthraquinone	-	-	-	-
Steroids	+	+	+	-
	Cardiac glycosides			
Legal test	+	+	+	+
Keller kiliani test	+	+	+	+
Salkwoski test	+	+	+	+
Lieberman test	+	+	+	+

Table 5. Quantitative Phytochemical Constituents of *V. amygdalina* Extracts.

Phytochemical	**Hot water extract**	**Methanol extract**	**Cold water extract**	**Ethyl acetate extract**
Flavonoid (mg/g)	1.40±0.01^{a}	2.07±0.02^{c}	1.47±0.01^{b}	1.38±0.22^{a}
Terpenoid (mg/g)	9.70±0.04^{b}	23.56±0.05^{d}	13.51±0.06^{c}	7.74±0.08^{a}
Tannin (mg/g)	3.26±0.00^{b}	4.33±0.00^{d}	3.74±0.04^{c}	3.00±0.09^{a}
Alkaloids (mg/g)	0.00±0.00^{a}	3.22±0.29^{b}	0.00±0.00^{a}	3.80±0.05^{c}
Saponin (mg/g)	35.33±0.27^{a}	85.19±0.25^{d}	58.09±0.16^{b}	82.17±0.97^{c}
Steroid (mg/g)	8.57±0.00^{b}	10.86±0.05^{d}	9.62±0.00^{c}	0.00±0.00^{a}
Glycoside (mg/g)	19.18±0.01^{b}	36.06±0.05^{d}	26.99±0.05^{c}	15.10±0.08^{a}

Values are presented as mean ± SE of triplicates, values in the same row carrying the same superscript are not significantly different at $p < 0.05$ according to Duncan's New Multiple Range Test.

Table 6. Antibacterial Activity of Various Extract of *V. amygdalina* at 100 mg/ml.

S/N	Isolates	Cold water	Hot water	Methanol	Ethyl acetate	PC	NC
1	***S. aureus***	14.17±0.44^{a}	18.83±0.17^{b}	17.17±0.44^{b}	16.83±1.01^{b}	28.67±0.67^{c}	0.00
2	***K. pneumoniae***	16.17±0.44^{ab}	16.17±1.01^{ab}	17.83±1.01^{b}	14.17±0.73^{a}	26.83±0.17^{c}	0.00
3	***E. coli***	13.17±0.44^{b}	12.83±0.44^{b}	12.83±0.44^{b}	10.17±0.44^{a}	22.00±0.58^{c}	0.00
4	***S. epidermidis***	11.83±0.60^{b}	11.17±0.44^{ab}	9.83±0.73^{a}	10.17±0.44^{ab}	29.83±0.44^{c}	0.00
5	***P. aeruginosa***	11.17±0.60^{ab}	14.17±0.60^{c}	12.83±0.60^{bc}	10.33±0.33^{a}	12.00±0.58^{ab}	0.00
6	***P. mirabilis***	7.17±0.17^{a}	7.50±0.29^{a}	9.17±0.73^{b}	9.50±0.29^{b}	23.50±0.76^{c}	0.00

Values are presented as mean ± SE of triplicates, values in the same row carrying the same superscript are not significantly different at $p < 0.05$ according to Duncan's New Multiple Range Test.

Table 7. Antibacterial Activity of Various Extract of *V. amygdalina* at 200 mg/ml.

S/N	Isolates	Cold water	Hot water	Methanol	Ethyl acetate	PC	NC
1	***S. aureus***	16.00±0.58^{a}	21.00±0.58^{c}	17.67±0.88^{ab}	19.00±0.58^{bc}	28.67±0.67^{d}	0.00
2	***K. pneumoniae***	17.00±0.58^{a}	18.00±0.58^{a}	17.67±1.20^{a}	16.00±0.58^{a}	26.83±0.17^{b}	0.00
3	***E. coli***	14.00±0.58^{b}	13.33±0.88^{b}	13.00±0.58^{b}	10.00±0.58^{a}	22.00±0.58^{c}	0.00
4	***S. epidermidis***	12.83±0.44^{b}	12.17±0.73^{b}	10.17±0.44^{a}	11.83±0.44^{b}	29.83±0.44^{c}	0.00
5	***P. aeruginosa***	12.00±0.58^{a}	15.00±0.58^{b}	14.00±0.58^{b}	11.00±0.58^{a}	12.00±0.58^{a}	0.00
6	***P. mirabilis***	8.83±0.73^{a}	11.83±1.01^{b}	10.83±0.44^{ab}	11.83±0.44^{b}	23.50±0.76^{c}	0.00

Values are presented as mean ± SE of triplicates, values in the same row carrying the same superscript are not significantly different at $p < 0.05$ according to Duncan's New Multiple Range Test.

Table 8. Antibacterial Activity of Various Extract of *V. amygdalina* at 300 mg/ml.

S/N	Isolates	Cold water	Hot water	Methanol	Ethyl acetate	PC	NC
1	***S. aureus***	20.17±0.44^{a}	21.50±0.87^{ab}	21.50±0.76^{ab}	23.17±0.60b	28.67±0.67^{c}	0.00
2	***K. pneumonia***	22.00±1.15^{b}	20.17±0.44^{ab}	22.17±0.44^{b}	18.17±1.01^{a}	26.83±0.17^{c}	0.00
3	***E. coli***	15.17±0.60^{b}	14.33±0.33^{b}	14.63±0.72^{b}	12.17±0.44^{a}	22.00±0.58^{c}	0.00
4	***S. epidermidis***	13.33±0.88^{b}	13.50±0.76^{b}	11.00±0.58^{a}	12.50±0.50^{ab}	29.83±0.44^{c}	0.00
5	***P. aeruginosa***	12.83±0.60^{a}	15.97±0.26^{b}	15.50±0.29^{b}	12.50±0.50^{a}	12.00±0.58^{a}	0.00
6	***P. mirabilis***	10.50±0.87^{a}	13.17±0.17^{b}	13.50±0.29^{b}	13.83±0.60^{b}	23.50±0.76^{c}	0.00

Values are presented as mean ± SE of triplicates, values in the same row carrying the same superscript are not significantly different at $p < 0.05$ according to Duncan's New Multiple Range Test.

Table 9. Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) Value for Extracts of *V. amygdalina*

S/N	Isolates	Cold water extract (mg/ml)		Hot water extract (mg/ml)		Methanol extract (mg/ml)		Ethyl acetate extract (mg/ml)	
		MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
1	***S. aureus***	25	50	25	100	25	100	50	50
2	***K. pneumoniae***	50	100	12.5	25	25	25	50	200
3	***E. coli***	50	100	25	100	100	NF	50	100
4	***S. epidermidis***	25	50	50	100	100	100	50	100
5	***P. aeruginosa***	50	50	100	100	50	100	50	100
6	***P. mirabilis***	100	200	100	200	100	NF	50	100

Table 10. Compounds Detected in the Gas Chromatography - Mass Spectrometry of *V. amygdalina* Purified Ethyl Acetate Extract.

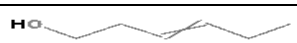
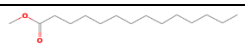
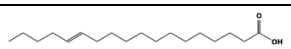
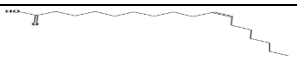
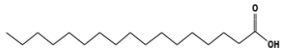
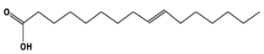
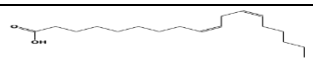
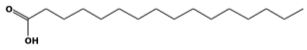
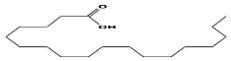
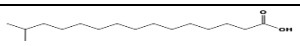
Peak ₦	RT	Compound detected	Mol. formular	Mol. weight	Peak area (%)	Comp. Wt (%)	Structures
1	10.00	3-Hexen-1-ol	$C_6H_{12}O$	100	1.76	2.31	
2	11.61	Cyclohexaneethanol	$C_8H_{16}O$	128	5.27	5.93	
3	17.46	9,12,15-Octadecatrienoic acid, (Z,Z,Z)-	$C_{12}H_{24}O_2$	200	11.18	10.21	
4	19.50	Methyl tetradecanoate	$C_{15}H_{30}O_2$	242	2.52	2.06	
5	20.50	Oleic acid	$C_{18}H_{34}O_2$	282	10.25	7.00	
6	22.00	trans-13-Octadecenoic acid	$C_{18}H_{34}O_2$	282	7.05	8.21	
7	23.50	cis-10-Heptadecenoic acid	$C_{17}H_{32}O_2$	268	4.41	1.04	
8	24.83	Heptadecanoic acid	$C_{17}H_{34}O_2$	270	20.01	18.96	
9	27.00	9-Hexadecenoic acid	$C_{16}H_{30}O_2$	254	6.17	4.47	
10	27.48	9,12-Octadecadienoic acid (Z,Z)-	$C_{18}H_{32}O_2$	280	16.74	6.50	
11	32.25	n-Hexadecanoic acid	$C_{14}H_{28}O_2$	256	3.08	4.38	
12	35.92	Octadecanoic acid	$C_{18}H_{36}O_2$	284	6.61	7.41	
13	43.50	14-methylpentadecanoic acid	$C_{16}H_{32}O_2$	256	3.97	3.12	

Figure 1. Distribution of Samples in Relation to Age and Sex.

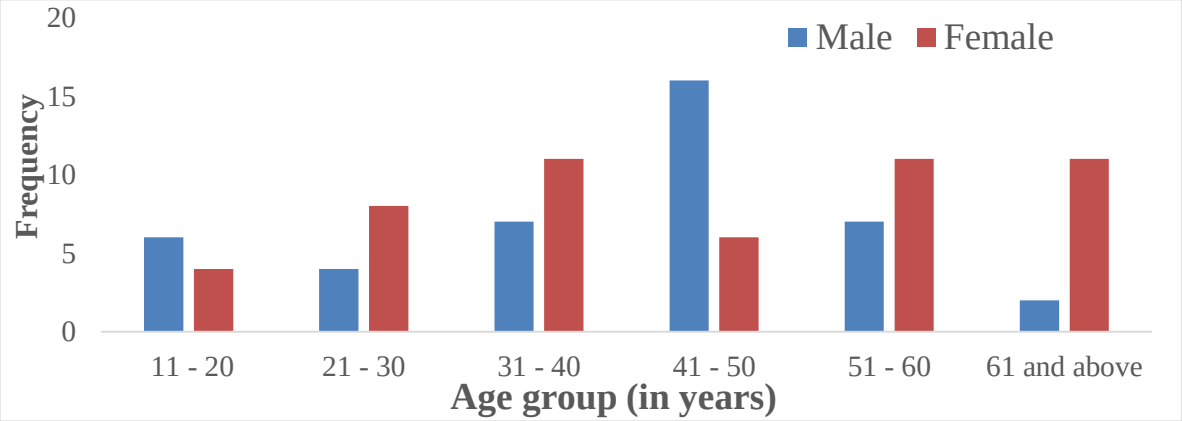


Figure 2. Percentage Occurrence of Bacteria Isolated from Wound Swab.

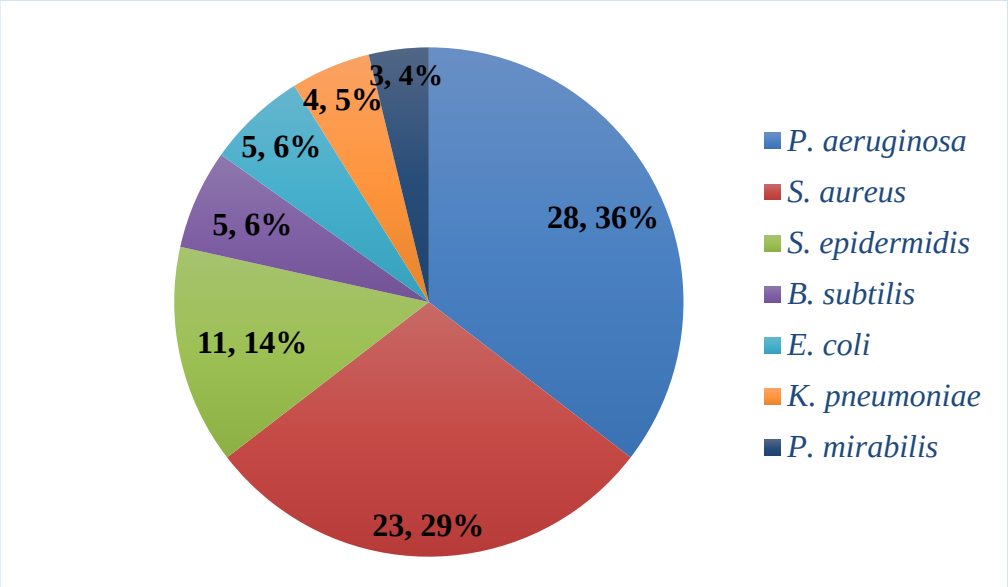
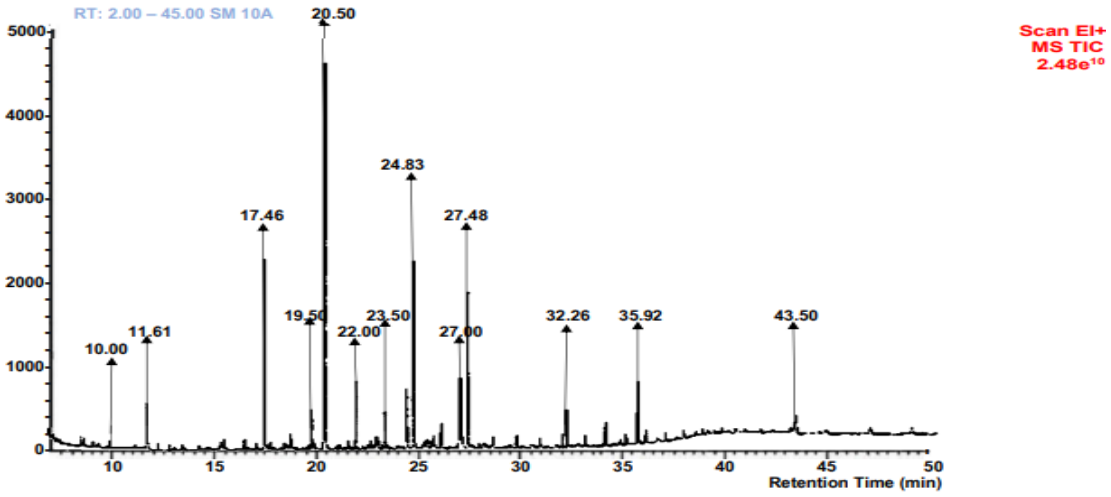


Figure 3. GC-MS Chromatographic Spectra of Ethyl Acetate Extract of *V. amygdalina*



.

Discussion

Bacterial wound contamination is a serious problem in the hospital and the treatment of wound infections remains a significant concern for surgeons. This study evaluated the distribution and antimicrobial resistance pattern of bacteria isolated from wound samples among patients attending University of Medical Sciences Teaching Hospital, Akure, Nigeria. In addition, the frequency of occurrence of these bacterial pathogens was estimated.

Patients with wound in this study were found to be mainly adults and the infection was more prevalent among females than males. The bacteria isolated from wound infection in this study are *Bacillus subtilis, Escherichia coli, Klebsiella pneumoniae, Proteus mirabilis, Pseudomonas aeruginosa, Staphylococcus aureus,* and *Staphylococcus epidermidis.* These bacteria are

known to be associated with wound infections. This is similar with a previous study by **Babayemi et al.** [26] who reported *S. aureus*, coagulase negative *S. aureus, E. coli, K. pneumoniae, P. mirabilis,* and *S. pyogenes* as the bacteria associated with wound infections. Another study by **Ijabani et al.** [18] reported *P. aeruginosa, S. aureus, S. epidermidis, B. subtilis, E. coli, K. pneumoniae, P. vulgaris* and *S. pyogenes* as the bacteria associated with wound infections in a specialist hospital in Jimeta Yola, Adamawa State, Nigeria.

P. aeruginosa was the most frequently occurring bacteria isolated from wound swabs in this study followed by *S. aureus*. Contrary to this study is the work previously done by **Ijabani et al.** [18] who reported *S. aureus* as the bacteria with the highest percentage occurrence in wound swabs of patients in Specialist Hospital, Yola, Adamawa State. A recent report by **Rashid et al.** [27] revealed that *P. aeruginosa* is one of the most common bacteria and is the cause of nosocomial infection and acquired drug resistance and it accounts for 11% of Hospital Acquired Infections and diseases, particularly in an immunocompromised individual.

A high level of resistance of the bacterial isolates to commonly used antibiotics was observed in this study. *Escherichia coli* were highly resistant to all antibiotics tested. *Staphylococcus aureus* was resistant to gentamycin. Amoxicillin and highly susceptible to streptomycin and septrin. This is in agreement with **Muhammad et al.** [28] who reported that *S. aureus* isolated from wounds were resistant to β-lactam antibiotics, macrolides and aminoglycosides). High rate of resistance demonstrated by the isolated bacteria to antibiotics may be due to practicing self-medication or unavailability of guidelines regarding the selection of drugs thereby leading to inappropriate use of antibiotics.

Phytochemicals such as saponins, tannins, terpenoids, flavonoids and cardiac glycosides were found present in the various extracts. A study by **Onifade et al.** [29] revealed the absence of alkaloids in the cold and hot water extracts of *V. amygdalina* which also conforms with the findings of this study. These phytochemicals have been shown to exhibit various pharmacological and biochemical actions and are responsible for the antimicrobial activity of the plant **Muhammed et al**. [30].

Stem bark extracts of *V. amygdalina* had antibacterial effects on the isolates at various concentrations. There was significant difference (at $p < 0.05$) in the activities of the various extracts against the bacterial isolates when the mean values of the zones of inhibition were compared.

Ethyl acetate and hot water extract of *V. amygdalina* used in this study possess higher antibacterial properties on *S. aureus* than cold water and methanol extracts. At 300 mg/ml concentration, the ethyl acetate extract mediated a zone of inhibition of 23.17±0.60 mm against *S. aureus* while *E. coli* has the lowest zone of 12.17±0.44 mm. The control was however more effective against the isolate with a mean zone of inhibition 28.67±0.67mm. Similarly, the control antibiotic mediated a wider zone of inhibition against the bacterial isolates. However, at 300mg/ml, the Hot water extract of *V. amygdalina* mediated a zone of inhibition of 15.97±0.26 against *P. aeruginosa* which is higher than the 12.00±0.58mm mediated by the control antibiotics. The extracts were least effective against *P. mirabilis* at the different concentrations. The methanol extract was more effective on *K. pneumoniae* with a zone of 17.83±1.01 at 100mg/ml and 22.17±0.44 at 300mg/ml. Similar findings by **Asfere et al.** [16] revealed the antibacterial activities of the methanolic extracts of the plant against *S. aureus*, and *P. aeruginosa.* Another work by **Tula et al.** [31] revealed that *E. coli* was not susceptible to the aqueous extract of *V. amygdalina* stem bark which is in contrary to the findings of this study. The antibacterial activity of *V amygdalina* was found to be dependent on the nature of the solvent used for extraction and the concentration of the extract. **Adetunji et al.** [32] reported that *V. amygdalina* at high concentrations and observable time limit could have a bactericidal effect of the extract on the organism.

The minimum inhibitory concentration (MIC) assay in this study reveals that ethyl acetate, methanol, hot water, and cold water extracts of *V. amygdalina* stem bark possess *in vitro* antibacterial activities at varying concentrations. The MBC values were higher than the MIC values in this work. This suggests that the extracts were bacteriostatic at lower concentrations and bactericidal at higher concentrations.

The GC-MS analysis of the purified ethyl acetate extract revealed the presence of Oleic acid which has been previously reported by **Rahdar et al.** [33] to have some antimicrobial properties, thus justifying why the plant exhibited antibacterial activities against the tested bacterial isolates. Other

compounds detected have also been previously reported to have antibacterial properties, and they include hexadecanoic acid, methyl ester, 9 – octadecenoic acid, 9,12 – octadecadienoic acid and palmitoleic acid [34].

Conclusion

This study revealed the presence of multiple antibiotic-resistant bacteria in wound infections. It further revealed that the different extracts of the stem bark of *V. amygdalina* used in this study possess some phytochemicals and bioactive compounds that make them good antibacterial agents against these multiple antibiotic resistant bacteria. Findings from this study have further established that *V. amygdalina* is a promising candidate and effective alternative treatment means for multiple antibiotic resistant bacteria (MARB) that are associated with wound infections.

Acknowledgments

The authors hereby acknowledge the Ondo State Ministry of Health for providing the ethical clearance for this study. Also, we acknowledge the University of Medical Sciences Teaching Hospital (UNIMEDTH), for allowing us take wound swab samples from patients in their premises.

Authors' contributions

AOO designed the study. DDA developed the methodology, acquired and analyzed the data and wrote the first draft of the manuscript. AOO and AKO both supervised the study. AKO reviewed and revised the manuscript. All authors read and approved the final draft of the manuscript.

Conflicts of interest

The authors have declared no conflict of interest.

Funding

No funding received.

References

1-**Mohammed ML, Arshan K, Jurla A, Khadse ST.** Isolation and identification of bacteria from wound infection and their antibiogram. International Journal of Technical Research and Science 2020; 5(4): 21-23.

2-**Mustafa HN, Al-Ogaidi I.** Efficacy of zinc sulfide-chitosan nanoparticles against bacterial diabetic wound infection. Iraqi journal of agricultural sciences 2023; 54(1): 1-17.

3-**Chizurum PC, George CN, Chibuzo VA, David UI, Emmanuel KA, Prisca CA**. Isolation and Identification of Bacteria from Wound Sepsis among Patients at Obigwe Orthopedic Clinic, Amorji Ubaha, Okigwe. South Asian Journal of Research in Microbiology 2022; 12(2): 9-15.

4-**Alaiya MA, Odeniyi MA.** Utilisation of Mangifera indica plant extracts and parts in antimicrobial formulations and as a pharmaceutical excipient: a review. Future Journal of Pharmaceutical Sciences 2023; 9(1): 29.

5-**Babatunde OJ, Okiti AF, Bayode MT, Babatunde SO, Olaniran, AM.** Antibiogram profile prediction of selected bacterial strains by *in silico* determination of acquired antimicrobial resistance genes from their whole-genome sequence. Bulletin of the National Research Centre 2022; 46(230): 1-7.

6-**Ogundare AO.** Antibacterial properties of the leaf extracts of *Vernonia amygdalina*, *Ocimum gratissimum*, *Corchorous olitorius* and *Manihot palmate*. Journal of Microbiology and Antimicrobials 2011; 3(4): 77-86.

7-**Bhavani T, Mohan RR, Mounica C, Nyamisha J, Krishna AG, Prabhavathi P.** Phytochemical screening & antimicrobial activity of *Ocimum gratissimum* review. Journal of Pharmacognosy and Phytochemistry 2019; 8(2): 76-79.

8-**Isunu LE, Omoya FO, Ajayi KO, Akharaiyi F, Ogundare AO, Babatunde OJ**. Evaluation of the antiplasmodial activities of methanol leaf extract of *Andrographis paniculata* (burm. f.): An *in vitro* and *in vivo* study. Microbes and Infectious Diseases 2023; 4(1); 296-303.

9-**Babatunde OJ, Ogundare AO, Adebolu TT.** Antibacterial activities of *Polyalthia longifolia* leaf extracts on multiple antibiotic-resistant bacteria isolated from hospital fomites in Akure,

Nigeria. Nusantara Bioscience 2023; 15(2): 149-160.

10-**Akinyele BJ, Oladejo, BO, Akinyemi AI, Ezem LO.** Comparative Study of the Antibacterial Effect of Mouth Washes and *Vernonia amygdalina* (Del.) on Some Tooth Decay Causing Bacteria. British Microbiology Research Journal 2014; 4(7): 749–758.

11-**Jarmai AH, Sheikh AM, Onyiche TE, Yunus H, Aji MA, Umar SM.** Antimicrobial Activity and Phytochemical Screening of Methanolic Leaf Extract of Vernonia amygdalina. South Asian Journal of Research in Microbiology 2022; 14(1): 23-35.

12-**Longe AO, Momoh JO, Asoro II.** Gas Chromatography-Mass Spectrophotometry (GC-MS) analysis of phytocomponents in the root, stem bark and leaf of *Vernonia amygdalina.* World Journal of Pharmaceutical Research 2017; 6(2): 35-49.

13-**Evbuomwan L, Chukwuka EP, Obazenu EI, Ilevbare L.** Antibacterial Activity of *Vernonia amygdalina* Leaf Extracts Against Multidrug Resistant Bacterial Isolates. Journal of Applied Sciences and Environmental Management 2018; 22(1): 17-21.

14-**Ruslim AK, Anitasari S, Ismail S, Oli EM, Yani S.** Effect of African leaves extract *Vernonia amygdalina* (Del.) on wound healing velocity after tooth extraction in Rattus norvegicus. Jurnal sains dan kesehatan 2017; 1(8): 408-414.

15-**Adesanoye OA, Ifezue AOC, Farombi EO.** Influence of chloramphenicol and amoxicillin on rat liver microsomal enzymes and lipid peroxidation. African Journal of Biomedical Research 2014; 17(3): 135-142.

16-**Asfere Y, Kebede A Muthuswamy M.** *In – Vitro* Antimicrobial Activities and Phytochemical Screening of *Calotropis procera* (Ait) and *Vernonia amygdalina* (Del.) Extracts Against Some Medically Important Pathogenic Bacteria. American Journal of Bioscience and Bioengineering 2018; 6(6): 42-45.

17-**Cheesbrough M.** Biochemical test to identify bacteria. District Laboratory Practice in Tropical Countries 2nd Edition 2006; 63-70: 136-138.

18-**Ijabani E, Salihu A, Pola BJ, Shitu A.** Antibiogram and Plasmid Mediated Resistance in Bacteria Isolated from Infected Wounds. South Asian Journal of Research in Microbiology 2022; 12(3): 1-12.

19-**Isunu LE, Omoya FO, Ogundare AO, Babatunde OJ, Bayode MT, Ajayi KO.** Antibacterial activity of *Andrographis paniculata* (burm. F.) Methanol leaf extract on bacteria consortia isolated from blood of diabetic patients. Bacterial Empire 2022; 5(1): e381.

20-**Clinical and Laboratory Standards Institute (CLSI).** Performance standards for antimicrobial susceptibility testing: 30th informational supplement M100-S20. Clinical Laboratory Standards Institute 2020, Wayne, P. A., USA.

21-**Gopu C, Chirumamilla P, Daravath SB, Shasthree T, Vankudoth S.** GC-MS analysis of bioactive compounds in the plant parts of methanolic extracts of *Momordica cymbalaria* Fenzl. Journal of medicinal plant studies 2021; 9(3): 209 – 218.

22-**Trease GE, Evans WC.** *Pharmacognosy*. Eleventh Edition. Brailliar Trind Can. Macmillan Publishers, London 1989.

23-**Sofowora A.** Medicinal plants and traditional medicine in Africa. Spectrum books Ltd. Ibadan, Nigeria 1993; P. 289.

24-**Harborne JB.** Phytochemical methods: A guide to modern techniques of plant analysis. 3rd Edition. Chapman and Hill, London 1998; 279.

25-**Kone JK, Bello OO, Onifade AK.** Antimicrobial potency of *Euphorbia*

heterophylla against selected clinical isolates. The proceedings of the Nigerian academy of science 2020; 13(2): 20-32.

26-Babayemi OO, Oke EA, Bayode MT. Antibacterial activity of *Jatropha tanjorensis* leaf extracts against bacteria associated with wound infections from the clinical setting. Nusantara Bioscience 2021; 13(2) 239-246.

27-Rashid MA, Mansour HN. Study of Antibiotic Resistant Genes in *Pseudomonas aeruginosa* isolated from burns and wounds. Archives of razi Institute 2022; 77(1): 403 – 411.

28-Muhammad A, Abubakar U, Zage IA, Lawan D, Fagwalawa. *In Vitro* Antibacterial Activity and Phytochemical Screening of *Garcinia Kola* Extracts against Methicillin Resistant *Staphylococcus Aureus* (MRSA). Journal of Pharmacy and Pharmaceutics 2018; 5(1): 13- 18.

***29*-Onifade AK, Agunloye OO.** Antibacterial Assessment of Crude and fractionated Extracts of *Vernonia amygdalina* Leaf against Multiple Antibiotic Resistant Bacteria of Wound Infection. Asian Journal of Research in Medical and Pharmaceutical Sciences 2019; 7(1): 1-12.

30-Muhammed AB, Doko HI, Ibrahim A, Abdullahi B, Yahaya H, Sharfadi RS. Studies on inhibitory effects of extract of *Vernonia amygdalina* used in traditional poultry farming against some bacteria isolated from poultry droppings. Scholars Journal of Applied Medical Sciences 2014; 2(6E): 3185-3192.

31-Tula MY, Azih AV, Iruolaje FO, Okojie RO, Elimian KO, Toy BD. Systematic study on comparing phytochemicals and the antimicrobial activities from different parts of *V. amygdalina.* African Journal of Microbiology Research 2012; 6(43): 7089-7093.

32-Adetunji CO, Olaniyi OO, Ogunkunle ATJ. Bacterial Activity of Crude Extracts of Vernonia amygdalina on Clinical Isolates. Journal of Microbiology and Antimicrobials 2013; 5(6): 60– 64.

33-Rahdar A, Beyzaei H, Saadat M, Yu X, Trant JF. Synthesis, physical characterization, and antifungal and antibacterial activities of oleic acid capped nanomagnetite and cobalt-doped nanomagnetite. Canadian Journal of Chemistry 2020; 98(1): 34-39.

34-Watanabe T, Yano S, Kawai T, Jinbo Y, Nonomura Y. Selective antibacterial activity of palmitoleic acid in emulsions and other formulations. Journal of Surfactants and Detergents 2021; 24(6): 973-979.