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Antibacterial activity of ethanol and methanol extract of *Vernonia amygdalina, Ocimum gratissimum* and *Aloe barbadensis* against *Pseudomonas aeruginosa* and *Staphylococcus aureus* isolated from wound infected patients

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Abstract
Introduction: Wound infection is debilitating to patients and a major cause of prolong hospitalization due to the increasing failure of antibiotics treatment. With the ever-increasing emergence of multiple antibiotics resistance among wound isolates, there is a limited therapeutics option for such bacteria strains thus making the need for exploring possible alternatives plant-based antibacterial agent a necessity. This study was aimed to determine the antibacterial activity of ethanol and methanol extract of *V. amygdalina, O. gratissimum* and *A. barbadensis* against wound isolates of *S. aureus* and *P. aeruginosa.*
Materials and Methods: A total of two hundred (200) infected wound sample were collected and analyzed using Standard microbiological techniques for identification of *S. aureus* and *P. aeruginosa.* The Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal concentration (MBC) of the plant extracts on the test isolates was determine using Agar well diffusion method.
Results and Conclusion: The infected wound sample yield 21 (10.5%) and 14 (7.0%) *S. aureus* and *P. aeruginosa* respectively. The methanolic extract of *V. amgydalina* showed antimicrobial activity with the mean inhibitory zone diameter of 10-22 mm against *S. aureus* and 8-18mm against *P. aeruginosa.* Ethanolic extract of *V. amgydalina* was also found to be potent and had antimicrobial activity against the test organism with the mean inhibitory zone diameter (IZD) of 6-18mm against *S. aureus* and 6-16mm against *P. aeruginosa* respectively. Both methanolic and ethanolic extracts of *V. amygdalina* was found to be more potent, inhibiting all the bacterial isolates, thus, showing higher antimicrobial activity compared to the methanolic and ethanolic extracts of *O. gratissimum* with the peak mean inhibitory zone diameter of 2-10mm against *S. aureus* and 2-4mm against *P. aeruginosa* in contrast to the control drug (ciprofloxacin) with mean inhibition zone diameter of 12mm. The crude extracts of methanolic and ethanolic of *A. barbadensis* also showed minimal antimicrobial activity with mean inhibition zone diameter of 2-6mm and 2-4mm against *S. aureus* and 2-4mm and 2mm against *P. aeruginosa*. The minimum inhibitory concentration and minimum bactericidal concentration of crude extracts of *V. amygdalina* on the test organism were 6.25mg/ml against both *S. aureus* and *P. aeruginosa*. The minimum inhibitory concentration and minimum bactericidal concentration of both methanol and ethanol extracts of *O. gratissimum* ranged between 25mg/ml - 50mg/ml against *S. aureus* while, the minimum inhibitory concentration and minimum bactericidal concentration of methanol and ethanol crude extracts of *A. barbadensis* against test organisms also increased in the following order; ethanol < methanol. Our study report the *in-vitro* antibacterial activities of *V. amygdalina* extracts, the leaf extracts efficacy could ameliorate or enhance *in-vivo* wound healing and could be used as broad spectrum antibiotics in the management of wound infections owing to it phytomedicinal properties. *A. barbadensis* and *O. gratissimum* were effective at higher concentration, which indicate medicinal potential that requires further exploration of the plant extracts in wound infection treatments, considering their antimicrobial effects on bacterial isolates.

Keywords: *Staphylococcus aureus, Pseudomonas aeruginosa, V. amygdalina, A. barbadensis, O. gratissimum, antibacterial*

Introduction
Staphylococcus aureus and *Pseudomonas aeruginosa* are the major primary pathogens implicated in wound infection [1, 2]. The bacteria may gain access to the wound through the environment, beddings, dressings, the patient's body fluids, or the hands of the patient or

health-care provider, [2, 4]. *S. aureus* and *Pseudomonas aeruginosa* are cause of hospital acquired (nosocomial) infection of surgical wounds and infections associated with indwelling medical devices [1, 5, 6, 7]. The bacterial expression of virulence, proliferation at the site of infected wound and antibiotic resistance intensifies the severity of infections which leads to prolong hospitalization and delay wound healing process with excruciating discomfort [1, 8]. Treatment of infected wounds using conventional antibiotics has been so problematic worldwide owing to the insurgence of antibiotic resistant bacterial strains [1, 9]. Earlier report has indicated the failure of most convectional antibiotic against infected wound bacteria as they are often multi-drug resistance causing limited therapeutic option [1]. With the increasing emergence of multiple antibiotics resistance, wound isolates are posing enormous public health concerns thus making the need for exploring possible alternatives plant-based antibacterial agent a necessity.

Some of these medicinal plants are *bitter leaf* (*V. amygdalina*), *Scent leaf (O. gratissimum), Aloe vera, Costus afer, Buchholzia coriacea* and *Psychotria microphylla* [10, 11, 12]. These plants contain phyto-ingredients which promotes quick healing of diseases like wound infections, cancer, lower blood sugar levels, relief of constipation [10, 11, 12]. In this sense, natural compounds from medicinal plants can ameliorate the innate immune response by stimulating cell proliferation and differentiation of keratinocytes and dermal fibroblasts, and can play an essential role in collagen synthesis which leads to the acceleration of the wounds healing process [10, 11, 12, 13].

This herbal treatment of diseases with little or no side effects is easily available at little or no cost. This could be the alternative means of arresting most cases of infection by pathogenic organisms.

Hence, our study revisit the alternative treatment methods using medicinal plants in the management of wound infections.

Materials and Methods

Study Area

This study was undertaken over a period of six months from May 2023 to November 2023. It was carried out at Alex-Ekwueme Federal Teaching Hospital Abakaliki, and is located at latitude 6.3231º N and longitude 8.1121ºE in Ebonyi State [14]. Ebonyi State is located in the South East geo-political zone of Nigeria. Alex-Ekwueme Federal University Teaching Hospital (AE-FEUTHA) is sited at the heart of Abakaliki, the capital of Ebonyi State. It is made up of two Annex, AE-FEUTHA 1, and AE-FEUTHA 2. The Alex Ekwueme Federal University Teaching Hospital Abakaliki was created by upgrading the former Federal Medical Centre Abakaliki to a Teaching Hospital in December 2011. The former Federal Medical Centre was built by Nigerian Federal Government in 1973. The Hospital is located along Uga Street, in Ebonyi Local Government Area, Ebonyi State. They have different admission wards including; the outpatient ward (GOPD), male and female surgical ward, male and female medical wards, pediatrics, ophthalmologic ward, labour ward, Orthopedic ward and gynecology etc.

Ethical Clearance and Research Criteria

Ethical clearance and consent with reference no: AEFUTHA/34/REC/023 were obtained from the ethical committee of the hospital management board of Alex-Ekwueme Federal University Teaching Hospital Abakaliki. Study criteria includes; wound samples from both outpatient and inpatients of the hospital with post-operative wound, surgical wound, burn wound infections were processed. Patients with infected wound but had received antibiotics in the last three months were excluded from the study while patients with wound infections who have not received antibiotics in the past three months were included in the study. However, the study did not interfere with normal management of the patients.

Sample Collection and bacteria isolation

The infected wound samples were aseptically collected using sterile swab sticks. Demographic information (sex, age, educational level and occupational status) of each patient was obtained and kept confidential. The patients were instructed not to apply any antiseptics or antibiotics on the wound before sample collection. Each of the collected swab samples was properly labeled and stored in ice packed flask. During bacteriological analysis the wound swabs were first inoculated into nutrient broth (Thermo Fisher Scientific, U.S.A) to enhance the growth of the organisms and incubated at 37 ºC for 18 -24 hours. After that, it was transferred aseptically using wire loop onto Cetrimide agar (bioMérieux, France) and Mannitol salt agar (bioMérieux, France) plates for the isolation of discrete colonies. Inoculated culture media plate were incubated at 37 ºC for 24 hours. Suspected and significant bacteria colonies were sub-cultured onto freshly prepared plates of Brain-Heart Infusion agar (Lifesave Biotech, USA) for the isolation of pure cultures of the organism of interest (*P. aeruginosa*, and *S. aureus*) [15]. Standard microbiological techniques for identification of pure culture strain was performed using Vitex 2 automated system (bioMérieux, France) [16].

Collection of Plant Samples: The plants were collected from botanical gardens within Doctors' Quarters at AE-FEUTHA. They were identified and assigned a voucher number by a taxonomist; Prof. C.A. Nnamani of Department of Applied Biology Ebonyi State University, Abakliki. The part of the plant that was used for this study was the leaf.

Preparation of Plant Material for Extraction

The different plants leaf obtained were washed properly with sterile water and allowed to drain out completely at room temperature for a week. When fully dried, they were ground into fine powder using ethanol cleaned manual grinder and sieved with net sieve [12].

Methanol extraction

The ground leaf powder of each plant was weighed 150g each and soak in 500 ml of methanol (BDH Chemicals Ltd, Poole England) respectively. This was kept in different conical flask with tight fitted cover and was properly agitated intermittently for 24 hours. It was placed over the plate and the extract was pour into it and pressed to separate the fiber from the juice. This was kept to evaporate and to dry under room temperature. After evaporation and dryness, it was collected and kept in different clean specimen bottles and labeled for future use [11].

Ethanol Extraction: As stated above, 150g of each dried leaf powder was soaked in 500ml of absolute ethanol

(Thermo Fisher Scientific, U.S.A). This was kept in conical flask with tight fluted cover and properly agitate at 2 hrs interval for 72 hrs. Other procedures as above was followed until complete evaporation was achieved, collected and kept in different specimen bottles and labeled accordingly [11, 12].

Screening for the Antibacterial activity of Ethanol and Methanol leaf extracts of *V. amygdalina, O. gratissimum* and *A. barbadensis* against the test bacteria

The antibacterial bioassay of *V. amygdalina, O. gratissimum* and *A. barbadensis* was performed using agar well diffusion technique as described by Peter *et al*. [12]; Nwankwo *et al*. [13]. The suspension of test isolates was streak evenly using a sterile swab on solidified Mueller-Hinton agar (Lifesave Biotech, USA) plates. A sterile cork borer (Supertek®, USA) was used to perforate five (5) well of 6 mm on a solidified Mueller-Hinton agar plate. Then 1g of plant extract yield from respective extraction solvents was subjected each to 10^{-5} serial dilution using 70% Dimethy lsulphoxide (Guangdang Guanghua Chemical Factory Co. Ltd, China) as a diluent. The extracts diluted concentrations of (200 mg/ml, 100mg/ml, 50 mg/ml, 25 mg/ml, 12.5 mg/ml and 6.25 mg/ml) was each used to fill each agar well plate aseptically and labeled respectively and incubate at 37 °C for 48 h. Zones of inhibition was measured using meter rule and recorded in millimeters. Agar well diffusion technique as described above was repeated for antibacterial activity of ethanol and methanol leaf extracts of *V. amygdalina, O. gratissimum* and *A. barbadensis*, against test isolates *in vitro*. The Minimum Inhibitory Concentration of the extract was determined by checking the lowest concentration of the extracts on agar plates that inhibit bacterial growth.

Standard overnight culture suspension of 0.5 McFarland or barium sulphate (Tianjin kermel Chemical Reagent Co., Ltd, China) solution equivalents of the tested organisms was inoculated on the surface of sterile Mueller-Hinton agar plates and allowed to stand for 30 minutes. A set of 6 single standard antibiotic disk was place aseptically on the inoculated Mueller - Hinton agar (Lifesave Biotech, USA) and was incubated at 37 °C for 24 hours and inhibition zone diameter was taken using a meter rule to nearest millimeter. Test isolates were classified as resistant, intermediate or susceptible based on the zone of inhibition following the standard interpretive chart [17] for Ciprofloxacin (5 µg) disc (Oxoid, Uk) used as positive control.

Determination of Minimum Inhibitory Concentration (MIC) of the Plant Leaf Extracts on Test Isolates

The Minimum Inhibitory Concentration (MIC) of the leaf extracts on the test isolates was determined by agar well diffusion method [18]. The millimeter (10 ml) volume of double strength method Mueller - Hinton agar (Lifesave Biotech, USA) at 45 °C was diluted with equal volume of the test extract in graded concentration (200, 100, 50, 25, 125, 6.25). These were poured aseptically into sterile petri-dishes and dried at 37 °C for 1hour with the lid slightly raised. Standardized test bacteria (10^6 cfu/ml) were aseptically inoculated on the surface of a sterile Mueller-Hinton agar (Lifesave Biotech, USA) for each concentration of the test plant extract. These were incubated at 37 °C value was taken as the least concentration of the extract showing no detectable growth [18].

Determination of Minimum Bactericidal Concentration (MBCs) of the Plant Leaf Extracts on test Isolates

The MBC of the plant extracts was determined by sub culturing portions of the agar from plates that showed no growth in the tests for determination of MICs. These agar portions were transferred respectively into plates containing freshly prepared MHA. These plates were incubated at 37 °C for 24 hrs and were observed for growth. The absence of growth at the end of incubation period signify total cell death. The minimum concentration of the plant extracts that produces total cell death was taken as the MBC [18].

Results

Socio-demographic Parameters of Wound Infected Patients in AE-FUTHA

The socio-demography of wound infected patients in AEFUTHA is shown in Table 1. It showed different parameter like age groups which ranged from 21-70, those within the age of 21-30 years (40.5%) were the highest participant I the research sex which comprised 60 (30.0%) males and 140 (70.0%) females, Educational level which cut across 48 (24.0%) patients with tertiary education level, 58 (29.0%) with secondary education, 109 (54.5%) with basic education and 5 (2.5%) patients who had no educational experience. The occupation among the 200 patients comprised of 57 (28.5%) traders, 80 (40.0%) students/ unemployed individuals, 20 (10.0%) civil servants, 17 (8.5%) artisans, 11 (5.5%) professional workers and 15 (7.5%) others with unrecognized occupation.

Table 1: Socio-demographic characteristics of patients in AE-FEUTHA

Age in years	No & % Frequency (n)
21-30	81 (40.5)
31-40	75 (37.5)
41-50	21(10.5)
51-60	9 (4.5)
61-70	14 (7.0)
Total	200
Sex	
Male	60 (30.0)
Female	140 (70.0)
Total	200
Educational level	
Tertiary	48(24.0)
Secondary	58(29.0)
Primary	109(54.5)
None	5(2.5)
Occupational status	
Trading	57 (28,5)
Student/unemployed	80(40.0)
Civil servant	20(10.0)
Artisans	17(8.5)
Professionals	11(5.5)
Others	15(7.5)

Total 200

The distribution of bacteria isolated from wound samples of patients in AE-FETHA is shown in Table 2. It revealed that out of the 200 wound swab samples tested, a total of 21 (10.5%) yielded *S. aureus* while *P. aeruginosa* was isolated form a total of 14(70%) of the samples.

Table 2: Detection rate of bacteria/pathogen from wound samples of patients visiting AE-FEUTHA

S. N.	No. of samples	Isolated organism	No. of Positive	Detection rate (%)
1.	200	*S. aureus*	21	21 (10.5)
2.	200	*P. aeruginosa*	14	14 (7.0)
		Total		35 (17.5)

Antibacterial activity of Ethanol and Methanol leaf Extracts of *V. amygdalina* against *S. aureus and P. aeruginosa* isolated from wound infections: The methanol leaf extracts of *V. amygdalina* activity is shown in table 6. It revealed that in contrast with the ciprofloxacin as the control drug, *S. aureus* and *P. aeruginosa* were highly inhibited at 200mg/ml concentrations, with inhibition zone diameter (IZD) up to 22mm for *S. aureus* and 18mm for *P. aeruginosa*. The Ethanol leaf extract of *V. amygdalina* inhibition activity is also shown in table 3. It also revealed that in contrast with ciprofloxacin as the control drug, both *S. aureus* and *P. aeruginosa* were highly inhibited at 200mg/ml concentrations, with the inhibition zone diameter (IZD) of 18mm for *S. aureus* and 16mm for *P. aeruginosa*. The MIC and MBC as shown in table 4, revealed that the methanol extracts of *V. amygdalina* had the MIC and MBC of 16.25mg/dl against *S. aureus* and 6.25mg/ml against *P. aeruginosa.* Meanwhile, the ethanol extract of *V. amygdalina* had the MIC and MBC of 6.25mg/dl against *S. aureus* and *P. aeruginosa* respectively (as shown in table 4).

Table 3: Ethanol and methanol leaf extracts of *V. amygdalina* inhibition activity against *S. aureus* and *P. aeruginosa* isolated from wound infections

Concentrations (mg/ml)	Bacterial Isolates	Ethanol (IZD)	Methanol (IZD)	Ciprofloxacin (5 µg)	Distilled water
200	*S. aureus*	18	22	12	0
100	*S. aureus*	14	18	12	0
50	*S. aureus*	14	16	12	0
25	*S. aureus*	12	14	12	0
12.5	*S. aureus*	10	12	12	0
6.25	*S. aureus*	8	10	12	0
200	*P. aeruginosa*	16	18	14	0
100	*P. aeruginosa*	14	16	14	0
50	*P. aeruginosa*	12	14	14	0
25	*P. aeruginosa*	10	12	14	0
12.5	*P. aeruginosa*	8	10	14	0
6.25	*P. aeruginosa*	6	8	14	0

Key: 0 = Resistant, Ciprofloxacin (Positive control), Distilled water = Negative control

Table 4: MIC and MBC of the extracts (*V. amygdalina*) against *S. aureus* and *P. aeruginosa*

Bacterial Isolates	Ethanol		Methanol	
	MIC (Mg/ml)	MBC (Mg/ml)	MIC (mg/ml)	MBC (mg/ml)
S. aureus	6.25	6.25	6.25	6.25
P. aeruginosa	6.25	6.25	6.25	6.25

Key: MIC=Minimum Inhibitory Concentration, MBC=Minimum Bactericidal Concentration

The ethanol leaf extract of *O. gratissimum* inhibition activity is shown in table 5. It revealed that in contrast with the ciprofloxacin as the control drug, *S. aureus* and *P. aeruginosa* were inhibited at 200mg/ml concentrations, with a low inhibition zone diameter (IZD) up to 8 mm for *S. aureus* and 4mm for *P. aeruginosa*.

The methanol leaf extract of *O. gratissimum* inhibition activity also shows low inhibition activity in contrast with ciprofloxacin as the control drug having inhibition up to 12 mm. *S. aureus* had inhibition zone diameter (IZD) peaked at 10mm and *P. aeruginosa* had 6 mm.

The MIC and MBC as shown in table 6 ethanol extracts of *O. gratissimum* had the MIC and MBC of 25mg/ml against *S. aureus* and 100 mg/ml against *P. aeruginosa*. Meanwhile, the methanol extracts of *O. gratissimum* had the MIC and MBC of 12.5 mg/ml against *S. aureus* and 50 mg/ml against *P. aeruginosa* (as shown in table 6).

Table 5: Ethanol and Methanol leaf Extracts of *O. gratissimum* Inhibition activity against *S. aureus and P. aeruginosa* isolated from wound infections

Concentrations (mg/ml)	Bacterial Isolates	Ethanol (IZD)	Methanol (IZD)	Ciprofloxacin (5 µg)	Distilled water
200	*S. aureus*	8	10	12	0
100	*S. aureus*	6	8	12	0
50	*S. aureus*	4	6	12	0
25	*S. aureus*	2	4	12	0
12.5	*S. aureus*	0	2	12	0
6.25	*S. aureus*	0	0	12	0
200	*P. aeruginosa*	4	6	14	0
100	*P. aeruginosa*	2	4	14	0
50	*P. aeruginosa*	0	2	14	0
25	*P. aeruginosa*	0	0	14	0
12.5	*P. aeruginosa*	0	0	14	0
6.25	*P. aeruginosa*	0	0	14	0

Key: 0 = Resistant, Ciprofloxacin (Positive control), Distilled water = Negative control

Table 6: MIC and MBC of *O. gratissimum* extracts against *S. aureus* and *P. aeruginosa*

Bacterial Isolates	Ethanol		Methanol	
	MIC (Mg/ml)	MBC (Mg/ml)	MIC (mg/ml)	MBC (mg/ml)
S. aureus	25	25	12.5	12.5
P. aeruginosa	100	100	50	50

Key: MIC=Minimum Inhibitory Concentration, MBC=Minimum Bactericidal Concentration

Inhibition activity of Ethanol and Methanol leaf Extracts of *A. barbadensis* against *S. aureus and P. aeruginosa* isolated from wound infections

The ethanol leaf extract of *A. barbadensis* inhibition activity is shown in Table 7. It revealed that in contrast with the ciprofloxacin as the control drug, *S. aureus* and *P. aeruginosa* were inhibited at 200mg/ml concentrations, with a very low IZD up to 4mm for *S. aureus* and 2mm for *P. aeruginosa.* The methanol leaf extract of *A. barbadensis* inhibition activity also shows little inhibition activity of the extract against both *S. aureus* and *P. aeruginosa.* In contrast with the control drug having inhibition up to 12 mm and 14mm for *S. aureus* and *P. aeruginosa* respectively.

The MIC and MBC as shown revealed that the ethanol extracts of *A. barbadensis* had the MIC and MBC 100mg/ml against *S. aureus* and 200mg/ml against *P. aeruginosa.* Meanwhile, the methanol extracts of *A. barbadensis* had the MIC and MBC of 50mg/ml against *S. aureus* and 100mg/ml against *P. aeruginosa* (as shown in Table 8).

Table 7: Ethanol and Methanol leaf Extracts of *A. barbadensis* Inhibition activity against *S. aureus* and *P. aeruginosa* isolated from wound infections.

Concentrations (mg/ml)	Bacterial Isolates	Ethanol (IZD)	Methanol (IZD)	Ciprofloxacin (5 µg)	Distilled water
200	*S. aureus*	4	6	12	0
100	*S. aureus*	2	4	12	0
50	*S. aureus*	0	2	12	0
25	*S. aureus*	0	0	12	0
12.5	*S. aureus*	0	0	12	0
6.25	*S. aureus*	0	0	12	0
200	*P. aeruginosa*	2	4	14	0
100	*P. aeruginosa*	0	2	14	0
50	*P. aeruginosa*	0	0	14	0
25	*P. aeruginosa*	0	0	14	0
12.5	*P. aeruginosa*	0	0	14	0
6.25	*P. aeruginosa*	0	0	14	0

Key: 0 = Resistant, CIP = Ciprofloxacin (Positive control), Distilled water = Negative control

Table 8: MIC and MBC of leaf extracts of *A. barbadensis* against *S. aureus* and *P. aeruginosa*

Bacterial Isolates	Ethanol		Methanol	
	MIC (Mg/ml)	MBC Mg/ml)	MIC (mg/ml)	MBC (mg/ml)
S. aureus	100	100	50	50
P. aeruginosa	200	200	100	100

Key: MIC=Minimum Inhibitory Concentration, MBC=Minimum Bactericidal Concentration

Discussion

Several cultures have utilized plant in traditional medicine to treat a variety of ailments. Preparations of the plant include confection, lotion, and juice.

In our present study, ethanol and methanol leaf extracts of *V. amygdalina* was effective against *S. aureus* and *P. aeruginosa*. The clinical isolates of *S. aureus* was found to be the most susceptible isolate to methanol crude extract of *V. amygdalina* with an inhibition zone diameter range of 22 mm at 200 mg/ml concentrations followed by *P. aerugeinosa* with an inhibition zone diameter range of 18mm. The ethanol crude extracts of *V. amygdalina* also showed greater antimicrobial activity with inhibition zone diameter (IZD) range of 18mm at 200mg/ml concentrations against *S. aureus* and 16mm against *P. aeruginosa.* The MIC and MBC of *V. amygdalina* from our findings reiterate with earlier report by other researchers [18, 19, 20, 21]. The extract ability to inhibit bacterial growth, makes *V. amygdalina's* a viable herb for drug development. Because of its distinct bitter flavor and taste, *Vernonia amygdalina* is a significant medicinal plant with potent antibacterial, anticancer, antimalarial, and antiparasitic properties that grows widely throughout West Africa.

Although the inhibitory effect of ethanol and methanol leaf extracts was higher against Gram-positive *S. aureus* when compare with *P. aeruginosa.* Moreover, differences in the susceptibility of Gram positive and Gram negative bacteria might have been caused by differences in cell walls component of Gram positive and negative bacteria. *P. aeruginosa* possess intrinsic resistant to most antibacterial treatments and the bacteria cellular component may have reduce the penetration of the extract phytomolecule into the bacteria cell.

S. aureus and *P. aeruginosa* were inhibited at 200 mg/ml concentrations, with a very low IZD up to 4 mm for *S. aureus* and 2 mm for *P. aeruginosa.* The MIC and MBC as shown revealed that the ethanol extracts of *A. barbadensis* had the MIC and MBC 100mg/ml against *S. aureus* and 200 mg/ml against *P. aeruginosa*.

Various *A. barbadensis* antibacterial effects by different extraction methods were reported by others; in agreement with the results of the current study, Nejatzadeh-Barandozi and Enferadi [22] reported the skin extracts as a source of antibacterial agents against *S. aureus* and MRSA. The alcoholic extracts was reported as a stronger antibacterial and antifungal agent than aqueous extract [23] and the

methanolic *A. barbadensis* gel extract had more antibacterial activity against gram-positive bacteria compared to gram-negative bacteria [24].

Another researcher study conducted to check the antibacterial activity of ethanol extract of *A. barbadensis*, exhibited maximum inhibition against *S. aureus* [25]. The Aloe vera gel was found to have antibacterial activity against the test isolates with MIC and MBC values of 25 µg/mL and 50 µg/mL against MDR S. aureus 50 µg/mL and 100 µg/mL against MDR P. aeruginosa respectively [26]. Kaithwas *et al.* [27] showed that the *A. barbadensis* gel is rich in variety metabolites, such as, anthraquinone, polysaccharides, glycoproteins, glycosides, gamma-linolenic acid and prostaglandins which are effective against gram-positive bacteria in particular against *S. aureus*.

The ethanol and methanol leaf extract of *A. barbadensis* had no inhibition activity at 50 mg/ml, 25 mg/ml, 12.5 mg/ml and 6.25 mg/ml on both *S. aureus* and *P. aeruginosa.* This observation could substantiate the fact that the whole leaf extract component of *A. Vera*, might have contained trace quantity of anthraquinones and saponins [28] which are proven phytomolecules with antibacterial activities and were not sufficient in the extract to exact bacteriostatic or bactericidal effect.

Ethanol and methanol extracts of *O. gratissimum* were effective at 200 mg/ml, and 100mg/ml. Prior study reported Ethanol extracts of *O. gratissimum* showed more antibacterial activity against *S. aureus* over *E. coli* [29]. This result is in agreement to Agatemor [30] where it was reported that gram negative bacteria are more resistant than gram positive bacteria to the *O. gratissimum* oil which are antimicrobial agents. Nweze *et al.* [31] reported phytochemical screening of *O. gratissimum* in the presence of alkaloids, tannins, glycoside, saponin, resins, cardiac glycoside, steroidal terpenes and flavonoids. Flavonoids are reported to exhibit antioxidant activity.

Our investigation showed that most of the extract were ineffective at 25 mg/ml, 12.5 mg/ml and 6.25 mg/ml. This is could be due to variations in soil ecology, seasonal condition, plant age and the dissolution capacity of the different solvents which in turn affected the degree of phytochemicals extracted.

The ethanol extract had better inhibitory effect over methanol extract. This may be because the ethanol extract has the ability to denature the protein of the microbial cell of the isolates, thereby destroying the cell wall/membrane of the bacterial isolates. This observation may also be attributed to the higher volatility of the ethanol which tends to extract more active compounds from the samples than methanol.

The results obtained from this study, also indicates that the *O. gratissimum* and *A. barbadensis* extracts showed lower antimicrobial activity than the conventional available antibiotic used which is not in line with the work of Selvamohan *et al.* [32] who reported that *A. barbadensis* extracts exhibited the strongest antimicrobial activity than the conventional available antibiotics.

This is possibly due to the degree of solubility of the active ingredient to dissolve in the solvent and all the sensitive extracts were more at higher concentration than lower concentration. Also, the comparison of the activity of *A. barbadensis* extract with conventional antibiotics, such as ciprofloxacin confirmed reports by other researchers [33, 34, 35] that conventional antibiotics are more active than plant extracts. The above findings pointed out that the higher the concentration of the extracts, the high the sensitivities of bacterial isolates to the extracts as showed by the increased size of inhibition zones diameter and this is in conformity with prior studies [12, 13].

Conclusion

Our study report the *in-vitro* antibacterial activities of *V. amygdalina* extracts, the leaf extracts efficacy could ameliorate or enhance *in vivo* wound healing and could be used as broad spectrum antibiotics in the management of wound infections owing to it phytomedicinal properties. *A. barbadensis* and *O. gratissimum* were effective at higher concentration, which indicate medicinal potential that requires further exploration of the plant extracts in wound infection treatments, considering their antimicrobial effects on bacterial isolates.

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