Antioxidant and Antibacterial Activities of *Terminalia mantaly* H. Perrier (Combretaceae) on Multidrug Resistant (MDR) Wound Pathogens

Idowu P.A.*[1], Olaiya C.O.[2] and Afolayan F.J.[1]

[1]*Department of Pharmaceutical Microbiology, Faculty of Pharmacy, and* [2]*Department of Biochemistry, Faculty of Basic Medical Sciences*[2]*, University of Ibadan.*

*Corresponding Author: Email: igboyega@yahoo.com; Tel: +2348033524399

ABSTRACT

Multidrug resistant (MDR) wound pathogens have rendered many antimicrobials ineffective in the treatment of infected wounds. Medicinal plants used to treat wound infections, especially in developing countries can furnish new and effective agents. Therefore, *Terminalia mantaly* used locally to treat infections was evaluated for antioxidant and antibacterial activities. Methanol extracts of leaves and roots were tested at 20 and 10mg/ml on thirty-two clinical isolates of wound bacteria (*Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and *Staphylococcus aureus*) from three Nigerian hospitals. Antibacterial activity and Minimum Inhibitory Concentration (MIC) were determined by agar well diffusion and agar dilution methods respectively. Antioxidant activities were evaluated by catalase, lipid peroxidation, hydrogen peroxide and DPPH free radical scavenging activities. The extracts showed good antibacterial activity with zones of inhibition between 12-35mm on all the tested pathogens with better activity on Gram–negative bacteria including the MDR strains. The MICs (and MBC) of leaf and root extracts were between 0.625-5mg/ml (1.25-10mg/ml) and 0.625-5mg/ml (1.25-5mg/ml) respectively. Leaf extract gave good antioxidant activity, better than root (comparable with ascorbic acid) of 73.77% inhibition on hydrogen peroxide scavenging, 76.36% inhibition on lipid peroxidation and 61.68% DPPH radical scavenging activities. Extracts of *Terminalia mantaly* showed good antioxidant and antibacterial activities, justifying the traditional uses of the plants. Further, the activity on MDR pathogens revealed the plant as a potential source of newer antibacterial agents for treating wound infections caused by MDR pathogens.

Keywords: Wound pathogens, Resistance, *Terminalia mantaly,* Antibacterial, Antioxidant

INTRODUCTION

Wound infection is one of the most common diseases in developing countries because of poor hygienic conditions [1]. Aerobic or facultative pathogenic bacteria such as *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Klebsiella* species, *Escherichia coli*, *Proteus* species and β-haemolytic *Streptococcus* species are often implicated and isolated from infected wounds [2]. The leading role of *S. aureus*, *P. aeruginosa* and *Klebsiella pneumoniae* in wound infection has been severally documented [3,4]. Wound infections still represent an important cause of morbidity and mortality among humans, especially in developing countries like Nigeria owing to the fact that most pathogens found in wound infections have developed resistance to antibiotics used in treatment [2,3]. Moreover, emergence of multi-drug resistant (MDR) organisms rendering multiple antibiotics ineffective has inevitably called for alternative medicine in wound management. Medicinal plants have been used since time immemorial for treatment of various ailments of skin and dermatological disorders especially cuts,

wounds and burns [5]. Free radicals and oxidative reaction products have been implicated in tissue damage and chronic wound infections; however, antioxidants help to quench and scavenge those reactive species from the body systems, thereby protecting the body against their destructive effects [6,7]. Invariably, medicinal plants with antioxidant constituents are important to good health and wound healing. We are therefore in search of medicinal plants with good antibacterial activity especially on MDR pathogens and significant antioxidant power that can be explored for the treatment of infected wounds.

Terminalia mantaly H. Perrier (Combretaceae) called Madagascar almond or umbrella tree is a deciduous or evergreen tree with conspicuously layered branches and is reported to be native to Madagascar where it is of great ethnomedicinal importance; the bark and wood being used for treating diarrhoea and dysentery [8]. The plant is very abundant in Nigeria but it is used more as ornamental (called umbrella tree) than medicinal. Many *Terminalia* species, such as *T. chebula, T. catappa, T. superba, T. arjuna* and *T. glaucescens* are used medicinally [9], and an antibacterial agent was isolated from *T. ivorensis* [10]. In this study, we anticipated the role of *Terminalia mantaly* in the healing of infected wounds by investigating and reporting the antibacterial and antioxidant activities of methanol extracts of the leaves and roots on thirty-two clinical isolates of wound pathogenic bacteria.

MATERIALS AND METHODS

Plant materials

Terminalia mantaly leaves and roots were obtained from the University of Ibadan Botanical garden, Ibadan, Oyo state and was authenticated in Forestry Research Institute of Nigeria (FRIN), with a deposited voucher specimen number FHI-109992.

Extraction

Air dried parts of the plants were extracted with distilled methanol using a Soxhlet extractor. The extracted solutions were evaporated under reduced pressure to yield methanol extracts, which was dried at room temperature (20-25 ^{o}C) and stored at 4^{o}C for further uses.

Test organisms

Thirty-two (32) bacterial strains comprising *Staphylococcus aureus* (n=14), *Pseudomonas aeruginosa* (n=10) and *Klebsiella pneumoniae* (n=8) from wound infections were obtained from medical microbiology departments of University of Ilorin Teaching Hospital, Ilorin; Obafemi Awolowo Teaching Hospital, Ife and University College Hospital, Ibadan, Nigeria. *Staphylococcus aureus* (ATCC 25923) and *Pseudomonas aeruginosa* (ATCC 27583) used as reference standards were obtained from Department of Pharmaceutical Microbiology Laboratory, University of Ibadan. They were all maintained on agar slants at 4^{o}C prior to use.

Determination of Antibiogram

Antibiogram of the test isolates was determined by modified Kirby-Bauer disc diffusion method using a multi-disc containing the following antibiotics: for *Staphylococcus aureus* (Gram positive bacteria), amoxicillin, ofloxacin, streptomycin, ceftriaxone, gentamicin, pefloxacin, cotrimoxazole, ciprofloxacin, erythromycin, chloramphenicol, oxacillin, cefoxitin; for *Psedomonas aeruginosa* and *Klebsiella pneumoniae* (Gram negative bacteria), cefotaxime, ofloxacin, augmentin, nitrofurantoin, ciprofloxacin, ceftazidine, cefuroxime, gentamicin, imipenem and

ceftriazone. This was done with strict adherence to CLSI [11] guidelines.

Antimicrobial Assay of Extracts

Using agar-well diffusion method as described by Perez *et al.* [12], a 0.1ml of a two-fold dilution of overnight broth culture of each bacterium (corresponding to the turbidity of 0.5 McFarland standard of 1.0 x 10^8 cfu/ml) was seeded into nutrient broth. A sterile cork borer (diameter 8mm) was used to punch uniform wells on the set and dried agar. Each well was filled with 0.2ml of the crude extracts (20 and 10 mg/ml). Control wells containing gentamicin (10µg/ml) and 40% methanol were used as positive and negative controls respectively. A pre-incubation period of about 1hour at 4^0C was allowed for the diffusion of extracts and test solutions before incubation. Bacterial plates were incubated at 37^0C for 24hours after which the zones of inhibition were measured.

Determination of Minimum Inhibitory Concentration (MIC) of Plant Extracts

The minimum inhibitory concentrations of the plant's methanol extracts were determined by the agar dilution method. The plant extracts were prepared in graduated decreasing concentrations (from 20.0 to 0.08 mg/ml) and seeded into Mueller Hinton broth until it produces turbidity equal to the 0.5 McFarland standard No.1 from which the organisms were then streaked on each plate. All plates were incubated at 37^0C for 24 hours. The MIC was taken as the minimum concentration of plant extracts that inhibited discernible bacterial growth in the plates.

Determination of Minimum Bactericidal Concentration (MBC) of Plant Extracts

The MBC was determined for all the isolates by sub-culturing from all the test mixtures that failed to show growth in the tube of MIC on Nutrient agar plates using a sterile wire-loop and incubated at 37^0C for 24hours. The MBC was recorded as the lowest concentration of extracts inhibiting all growth [13].

DETERMINATION OF ANTIOXIDANT PROPERTIES

Catalase Activity

The activity of catalase (CAT) was measured according to the method of Aebi, [14] as the disappearance of hydrogen peroxide at 240nm in a reaction medium containing 1800µl of 50mM phosphate buffer (pH 7.0). 180µl of 300mM H_2O_2, and 20 µl of methanol extract (1:50 dilution). The reaction was monitored for 2minutes (10 intervals), at 240nm using a UV-visible spectrophotometer (Cecil CE 7200 spectrophotometer, Milton Technical Centre, England) and expressed as µmol of H_2O_2 consumed/min/mg of extract).

DPPH Radical Scavenging Activity

The methanol extract (100 µl, 1mg/ml) was added to 3.9ml of DPPH solution (0.025g/L) and the reactants were incubated at 25^oC for 30minutes. Instead of extract, a positive control of Ascorbic acid (AsA) was used. The mixture was shaken and allowed to stand in the dark at room temperature for 35minutes. Blank methanol and ascorbic acid (25 - 0.78µg/ml) were treated in the same way and served as negative and positive controls respectively. Free radical scavenging activity was calculated from absorbance values at 517nm [15] using the UV-spectrophotometer (Cecil CE 7200 spectrophotometer, Cecil instrument limited, Milton Technical Centre, England). The percentage reduction of DPPH was calculated using the following equation: DPPH scavenging activity (%) = [(A0-A1)/A0] x 100. Where A0 = absorbance of negative control, A1 = absorbance of

different concentrations of extract or standard drug using the formula:

% Inhibition (radical scavenging %) = (Ac-As) /Ac×100,

where Ac= Absorbance of control, As = Absorbance of Sample.

Hydrogen Peroxide Radical Scavenging Activity

One millimetre of extract (250µg/ml) was mixed with 2.4ml of 0.1M phosphate buffer (pH 7.4) and then 0.6 of a 43Mm solution of H_20_2 in the same buffer were added [16]. After 40minutes, the absorbance of reaction mixture was taken at 230nm against a blank solution (phosphate buffer without H_20_2) using the UV-spectrophotometer (Cecil CE 7200 spectrophotometer, England). Percentage scavenging of H_20_2 was calculated with Ascorbic acid as control.

% inhibition (radical scavenging %) = (Ac-As) /Ac×100, where Ac= Absorbance of control, As = Absorbance of Sample.

Lipid Peroxidation Inhibition using Thiobarbituric Acid Method

Extracts (2ml) and standard solutions (2ml) of the extract were added to 1ml of 20% aqueous trichloroacetic acid and 2ml of 0.67% aqueous thiobarbituric acid. After boiling for 10minutes, the samples were cooled. The tubes were centrifuged at 3000rpm for 30minutes. Absorbance of the supernatant was measured at 532nm in a spectrophotometer [17] (Cecil CE 7200 spectrophotometer, England). Percentage scavenging of H_20_2 was calculated with ascorbic acid as control.

% inhibition (radical scavenging %) = (Ac-As) /Ac×100, where Ac= Absorbance of control, As = Absorbance of Sample.

RESULTS

The result of antibiogram study on the clinical isolates of wound pathogens, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Klebsiella pneumoniae*, showed that most of the organisms have become resistant to conventional antibacterial agents than the standard strains (Table 1). The *Staphylococcus aureus* isolates showed high resistance to β-Lactam antibiotics with 100% resistance to Augmentin and Oxacillin and 92% to Cefoxitin; but 35.7% to Ceftriaxone (Table 1.1). The *S. aureus* isolates showed resistance to the fluoroquinolones tested as follows: Ofloxacin 64%, Pefloxacin 57% and ciprofloxacin 50% (Table 1.1). The *Ps. aeruginosa* isolates were the most resistant among the three bacterial species tested, showed 100% resistant to Cefotaxime, Cefuroxime, Augmentin, Nitrofurantoin and Imipenem, while the least resistance was recorded against Ceftazidime (60%), Gentamicin. Ofloxacin (70%) and Ciprofloxacin (80%) (Table 1.2). The *Klebiella* isolates were the most susceptible, with highest susceptibility to Imipenem (62.5%), followed by Gentamicin (62.5%), Ofloxacin (37.5%) and Ciprofloxacin (25%) (Table 1.3).

Antibacterial screening of the methanol extracts demonstrated a potent broad-spectrum antibacterial activity on wound pathogens (*S. aureus, P. aeruginosa, K. pneumoniae*). Both concentrations (20mg/ml and 10mg/ml) of the methanol extracts of *T. mantaly* leaves and roots showed clear zones of inhibitions comparable to those shown by the positive controls (ceftriaxone and gentamicin). The diameters of zone of inhibition were between 15-30mm for 20mg/ml and 12-25mm for 10mg/ml. The zones of inhibition were well enough to equal the zone of inhibition recommended by CLSI, 2007 for sensitivity of antibiotics. The negative control didn't show any zone of inhibition indicating that it didn't add any inhibitory effect. The results of the susceptibility tests of the extracts on each group of isolates are found on Table 2.

The minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC) of the methanol extracts on each isolate were recorded. The MIC values of both TML and TMR methanol extracts ranged between 0.625-5mg/ml, while the MBC ranged from 0.625mg/ml to 10mg/ml on all the test isolates. Details can be found on Tables 3. The antioxidant activity of the leaf and root extracts along with the ascorbic acid (control standard) are represented by the bar chart of Figure 1, including lipid peroxidation, hydrogen peroxide and DPPH free-radical scavenging activities. The catalase test for antioxidant activity showed that the plant's root extract at 1.088 μMH_2O_2/min/mg produced more inhibition than the leaf extract at 0.922 μMH_2O_2/min/mg. The leaf showed good antioxidant activities comparable to ascorbic acid while the roots were less active generally but showed good catalase activity.

Table 1: ANTIBIOGRAM OF CLINICAL ISOLATES FROM WOUND INFECTION

Table 1.1: Interpretative result of antibiotics susceptibility pattern of S*taphylococcus aureus* Isolates

Isolates	AMX	OFL	STR	CHL	CEF	GEN	PEF	COT	CPX	ERY	OXA	FOX
S1	R	R	I	S	I	R	I	R	I	R	R	R
S2	R	R	R	R	I	R	I	R	R	R	R	R
S3	R	S	I	I	R	R	S	R	S	R	R	R
S4	R	R	R	I	I	R	I	R	I	R	R	R
S5	R	R	I	R	R	I	R	R	R	S	R	R
S6	R	R	R	R	I	R	R	I	R	R	R	R
S7	R	R	R	R	R	R	R	R	R	R	R	R
S8	R	R	R	R	I	R	R	R	R	R	R	R
S9	R	R	R	R	I	R	R	R	R	R	R	R
S10	R	I	S	I	S	R	R	R	I	R	R	R
S11	R	R	R	R	S	R	R	R	R	R	R	S
S12	R	S	S	R	R	R	I	R	I	R	R	R
S13	R	S	R	R	R	R	R	R	I	R	R	R
S14	R	S	S	S	S	S	S	S	I	R	R	R
ATCC	R	S	R	R	I	I	S	R	S	R	R	R
% S	0.0	28.6	21.4	14.3	21.4	7.1	14.3	7.1	7.1	7.1	0.0	7.1
% I	0.0	7.1	21.4	21.4	42.9	7.1	28.6	7.1	42.9	0.0	0.0	0.0
% R	100	64.3	57.2	64.3	35.7	85.7	57.1	85.8	50.0	92.9	100	92.9

Key: R=Resistant; I=Intermediate; S=Susceptible; ATCC= Staphylococcus aureus ATCC 25923 AMX=amoxicillin, OFL=ofloxacin, STR=streptomycin, CEF=ceftriaxone, GEN=gentamicin, PEF=pefloxacin, COT=cotrimoxazole, CPX=ciprofloxacin, ERY=erythromycin, CHL=chloramphenicol; OXA=oxacillin, FOX=cefoxitin

Table 1.2: Interpretative result of antibiotics susceptibility pattern of *Pseudomonas aeruginosa* Isolates

Isolates	CXM	OFL	AUG	NIT	CPR	CAZ	CRX	GEN	IMP
P1	R	S	R	R	S	S	R	S	R
P2	R	R	R	R	R	R	R	R	R
P3	R	R	R	R	R	R	R	R	R
P4	R	R	R	R	R	S	R	R	R
P5	R	R	R	R	R	S	R	R	R
P6	R	R	R	R	R	R	R	R	R
P7	R	R	R	R	R	R	R	R	R
P8	R	R	R	R	R	R	R	R	R
P9	R	R	R	R	R	R	R	R	R
P10	R	S	R	R	S	S	R	S	R
ATCC	R	S	S	S	S	R	S	S	R
% S	0.0	30.0	0.0	0.0	20.0	40.0	0.0	30.0	0.0
% I	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
% R	100	70.0	100	100	80.0	60.0	100	70.0	100

Key: R= Resistant, S= Susceptible CXM= cefotaxime, OFL= ofloxacin, AUG= augmentin, NIT= nitrofurantoin, CPR= ciprofloxacin, CAZ= ceftazidime, CRX= cefuroxime, GEN= gentamicin, IMP= Imipenem; ATCC= *Pseudomonas aeruginosa* ATCC 27583

Table 1.3: Interpretative result of antibiotics susceptibility pattern of *Klebsiella pneumonia isolates*

Isolates	CRX	CPR	NIT	AUG	OFL	CXM	GEN	CTR	IPM	CAZ
K2	R	R	R	R	I	R	R	R	S	R
K3	R	R	R	R	S	R	R	I	S	I
K4	R	I	S	S	S	I	S	I	S	S
K5	R	S	R	R	I	R	S	R	I	R
K6	R	I	I	R	I	R	S	R	R	R
K8	R	R	S	R	R	R	R	I	S	R
K16	R	S	R	R	S	R	S	R	S	S
K18	R	R	R	R	R	R	S	R	R	R
% S	0.0	25.0	25.0	12.5	37.5	0.0	62.5	0.0	62.5	25.0
% I	0.0	25.0	12.5	0.0	37.5	12.5	0.0	37.5	12.5	12.5
% R	100	50.0	62.5	87.5	25.0	87.5	37.5	62.5	25.0	62.5

Key: R=Resistant; I=Intermediate; S=Susceptible; CXM=cefotaxime; OFL=ofloxacin, AUG=augmentin; NIT=nitrofurantoin; CPR=ciprofloxacin, CAZ=ceftazidime; CRX=cefuroxime; GEN=gentamicin; IMP=imipenem; CTR= ceftriazone

Table 2: Antimicrobial activity of extracts on clinical isolates wound pathogens

	ZONES OF INHIBITION (mm) OF EXTRACTS AND CONTROLS					
Extracts	TML		TMR		Gentamicin	Methanol
Isolates	20mg/ml	10mg/ml	20mg/ml	10mg/ml	10 µg	40%
S1	22	18	22	20	23	-
S2	19	18	15	12	25	-
S3	16	15	20	18	24	-
S4	20	18	20	20	25	-
S5	20	15	21	16	15	-
S6	19	15	18	15	-	-
S7	18	18	20	16	-	-
S8	20	15	21	17	-	-
S9	20	18	20	18	-	-
S10	20	20	26	20	20	-
S11	18	18	26	22	32	-
S12	26	25	26	25	26	-
S13	18	15	23	19	30	-
S14	27	23	28	25	25	-
P1	22	18	22	18	23	-
P2	22	18	22	20	-	-
P3	22	18	24	20	-	-
P4	24	20	24	20	-	-
P5	27	22	23	18	24	-
P6	22	22	26	25	-	-
P7	23	20	24	22	-	-
P8	21	19	30	24	-	-
P9	25	17	22	19	-	-
P10	25	22	25	21	17	-
K2	15	12	20	18	20	-
K3	20	16	20	16	20	-
K4	22	16	21	16	20	-
K5	22	17	24	20	21	-
K6	19	19	16	16	14	-
K8	16	14	16	14	22	-
K16	20	16	20	20	20	-
K18	22	20	22	21	23	-

Keys: TM= *Terminalia mantaly* leaves, TMR= *Terminalia mantaly* roots, S= *Staphylococcus aureus*, P= *Pseudomonas aeruginosa*, K= *Klebsiella pneumoniae* isolates

Table 3: MIC and MBC of *Terminalia mantaly* leaf and root extracts on wound pathogens

ISOLATES	MIC (mg/ml)		MBC (mg/ml)	
	TML	TMR	TML	TMR
S1	5.00	5.00	5.00	5.00
S2	5.00	10.00	10.00	5.00
S3	5.00	10.00	10.00	5.00
S6	5.00	5.00	5.00	5.00
S7	5.00	5.00	5.00	2.50
S8	5.00	10.00	10.00	5.00
S9	5.00	5.00	10.00	2.50
S10	5.00	5.00	10.00	2.50
P1	1.25	5.00	5.00	1.25
P2	1.25	2.50	5.00	0.63
P3	1.25	2.50	2.50	0.63
P6	2.50	2.50	2.50	1.25
P7	2.50	5.00	5.00	1.25
P8	1.25	5.00	1.25	0.63
P9	1.25	2.50	1.25	0.63
P10	1.25	2.50	2.50	0.63
K3	0.63	2.50	1.25	1.25
K4	5.00	5.00	2.50	2.50
K5	0.63	2.50	2.50	1.25
K6	0.63	2.50	2.50	1.25
K8	5.00	5.00	5.00	5.00
K16	1.25	2.50	1.25	1.25
K18	0.63	1.25	1.25	1.25

Keys: TML= *Terminalia mantaly* leaves, TMR= *Terminalia mantaly* roots, S= *Staphylococcus aureus*, P= *Pseudomonas aeruginosa*, K= *Klebsiella pneumoniae* isolates

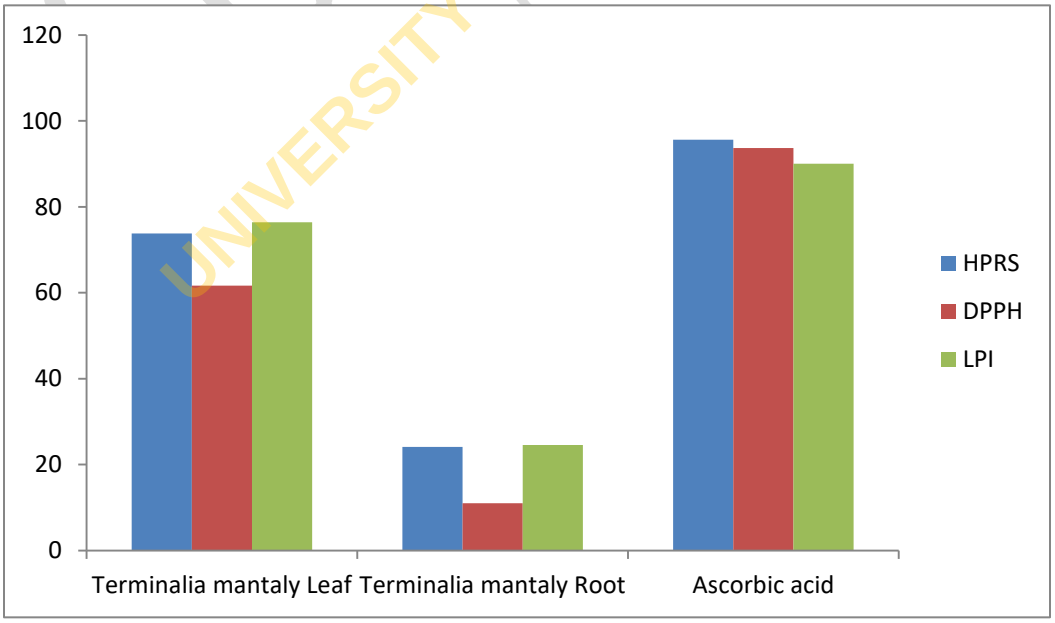

Fig. i: Result of antioxidant activity of Terminalia mantaly leaf and bark extracts

DISCUSSION

Bacterial contamination of wounds is an important cause of morbidity and mortality [2] and when such bacteria are multidrug resistant (MDR) in nature, the situation becomes critical and requires urgent interventions. Infection of wounds caused by surgery is a serious health risk, as studies have shown that 70% of the deaths of patients who have undergone surgery are caused by surgical site infections [18]. Many studies carried out in different parts of Nigeria showed that *P. aeruginosa*, *S. aureus*, *Klebsiella* sp., *E. coli* and *Proteus sp*. are the major pathogens associated with wound infections [2]. The leading role of *S. aureus* and *P. aeruginosa* in wound infection has been severally documented [3,4]. The wound pathogens, comprising *P. aeruginosa*, *S. aureus* and *K. pneumoniae*, used in this study were MDR as presented by the antibiogram results. Among the 14 strains of S. aureus tested, majority were resistant to β-lactam antimicrobials (amoxycilin 100%, oxacillin 100%, cefoxitin 92% and ceftriaxone 35.7%) which indicated that they may be MRSA, which are notorious leading causes of nosocomial infections [19]. The MRSA normally acquire resistance through the cassette containing the *mecA* gene that encodes the protein penicillin binding protein (PBP 2A) with low affinity for β-lactam antibiotics. Although they show some susceptibility to the fluoroquinolones (pefloxacin, ciprofloxacin and ofloxacin) and streptomycin, the resistance level was still very high. *Pseudomonas aeruginosa* encountered in this study displayed high level of resistance (100% to cefuroxime, augmentin, cefotaxime, imipenem and nitrofurantoin) which is an indication of MDR *Pseudomonas* that are resistant either intrinsically through the multidrug efflux pump or by acquisition through metallo-β-lactamase genes. Surprisingly, ceftazidime that was the most active drug, was active only on 40% of the isolates. In the case of the *Klebsiella* isolates, there was a high-level (62.5-100%) resistance to the β-lactams but susceptible to imipenem, gentamicin and fluoroquinolones in that order. *Klebsiella* have been implicated in MDR resistance through extended spectrum β-lactamase (ESBL) production. The antibiogram result is in agreement with previous reports [20,21,22].

The emergence of these multidrug resistance microbes has been attributed to the indiscriminate use of antibiotics over time. The irrational use of antibiotics without prescription and proper susceptibility test, overdosing, self-medication and prolonged hospital stay are all factors that have played significant roles in the emergence of these multidrug resistant pathogens in the third world countries [23].

However, it is interesting that *Terminalia mantaly* extracts showed a good activity on these MDR wound pathogens. The MIC values of the extracts obtained for the *S. aureus* test isolates (5mg/ml) were higher than that for the *K. pneumoniae* and *P. aeruginosa* isolates that ranged between 0.625 and 5mg/ml. The Gram–negative bacteria were more susceptible to the plant extracts than Gram–positive bacteria which contradict previous reports stating that plant extracts are more active against Gram–positive bacteria than Gram–negative bacteria [24,25]. It has been theorized that Gram–positive bacteria are more susceptible than Gram-negative bacteria due to the differences in their cell wall structure. Gram–negative organisms are considered to be more resistant due to their outer membrane acting as a barrier to many environmental substances, including antibiotics [26]. The plant's bioactive constituents may be more selective against Gram negative than Gram positive bacteria. The plant's extracts have shown good and

remarkable inhibitory and bactericidal activities on wound pathogens, similar to those reported on *Cassia fistula* [1] and *Moringa oleifera* [27]. The bactericidal activity of the plant's extract can be highlighted by calculating the MIC index (= MBC/MIC), which was found to be less than 4, thus confirming the bactericidal rather than bacteriostatic mechanism of antibacterial action.

The antioxidant potentials of the plant's extracts were also determined. It is believed that antioxidant activities of medicinal plants must be evaluated by more than one method (by at least two methods) in order to take into account different modes of action of a given antioxidant [28]. Factors like stereoselectivity of the radicals or the solubility of the extract in different testing systems have been reported to affect the capacity of extracts to react with and quench different radicals [29].

From the hydrogen peroxide radical scavenging activity using a concentration of 250µg/ml, *T. mantaly* leaves had the scavenging ability of 73.67% while roots had a scavenging activity of 24%. Hydrogen peroxide can sometimes give rise to hydroxyl radical in the cells and its over-expression will definitely delay wound healing. The phytoconstituents in *T. mantaly* leaf extract has shown that it has the highest capability to scavenge hydrogen peroxide radicals. It was observed that methanol extracts of leaves and roots had a DPPH scavenging activity of 61.68% and 11.04% respectively. The effect of antioxidants on DPPH is thought to be due to their hydrogen donating ability [30]. Though the DPPH radical scavenging abilities of the extracts were less than those of ascorbic acid at the same concentration, the study showed that the extracts have the proton-donating ability and could serve as free radical inhibitors or scavengers, acting possibly as primary antioxidants. The extracts inhibited lipid peroxidation; leaf extract had the inhibition percentage of 76.36% while that of roots was 24.55%. Phytochemicals present in the potent extracts has shown the ability to scavenge hydroxyl radical that reacts with the methylene groups of Polyunsaturated fatty acids (PUFA), the main components of membrane lipids, are susceptible to per-oxidation and thus preventing the aldehydes (final stable products of per-oxidation) to react with TBA to form thiobarbituric acid-malonaldehyde adduct with an absorbance maximum at 532nm. This led to reduction of adduct formation indicating their lipo-protective potential. This activity could be attributed to the hydroxyl radical scavenging by phytochemicals present in potent extracts. The catalase activity of the extracts showed that roots had higher capability to catalyse the dismutation of hydrogen peroxide in water and oxygen at a value of 1.088 µM of H_2O_2/min/mg extract than the leaves with the value of 0.922 µM of H_2O_2/min/mg extract. It is possible that the flavonoids present only in *T. mantaly* root were able to function effectively as antioxidant in catalase activity than in other methods used.

CONCLUSION

The extracts were active against both Gram-positive and Gram-negative bacteria isolates tested and this may indicate a broad spectrum of activity. The results of the study support the traditional application of *Terminalia mantaly* to treat infected wounds. The susceptibility of all the test organisms including the MDR bacterial pathogens goes further to prove that the plant has potentials as an alternative source of antimicrobial agents which can also overcome antimicrobial resistance. *Terminalia mantaly* leaf extract has also been revealed as a good source of antioxidant agents, highlighting its therapeutic potentials in the treatment of

MDR infected wounds. It is therefore recommended to isolate and characterize the antimicrobial (and antioxidant) agents from *Terminalia mantaly*.

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