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Evaluation of Antioxidant and Antibacterial Activities of Extracts of Celtis philippensis Blanco (Ulmaceae) on Multidrug Resistant Wound Pathogens

Philip A. Idowu[1]*, Faith O. Afolayan[1], Charles O. Olaiya[2]

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

***Corresponding author:**
Philip Adegboyega Idowu, E-mail: igboyega@yahoo.com Phone: +2348033524399

ABSTRACT

Background: Antimicrobial resistance among wound pathogens is increasing, creating the need to search for new and effective agents, especially among medicinal plants used to treat wounds traditionally. There is a worldwide search for medicinal plants with good antibacterial and antioxidant activities to treat infected wounds.

Objectives: *Celtis philippensis* used locally to treat infections was evaluated for its antioxidant and antibacterial activities against multidrug resistant (MDR) pathogens from infected wounds from three Nigerian hospitals.

Methods: Methanol extract of *C. philippensis* stem was tested at 20 and 10mg/ml on 32 wound pathogens comprising of *Staphylococcus aureus* (14), *Pseudomonas aeruginosa* (10) and *Klebsiella pneumoniae* (8). Agar diffusion methods was used to determine antibiogram and susceptibility to extracts while agar dilution method was used to determine minimum inhibitory concentrations (MIC) and minimum bactericidal concentrations (MBC). The antioxidant potential was evaluated by four methods: catalase activity, lipid peroxidation inhibition, hydrogen peroxide radical scavenging activity and DPPH radical scavenging activity.Statistical analysis was done using ANOVA.

Results: Antibiogram showed that the test organisms were MDR bacteria. The extract was active on all the tested pathogens with zones of inhibition of between 13-35mm. The MIC and MBC values ranged between 0.625-5mg/ml for all the test organisms and MIC index indicated bactericidal activity. The methanol extract showed good antioxidant activity (lipid peroxidation inhibition and catalase activity) comparable with ascorbic acid standard.

Conclusion: Antioxidant and antibacterial activities of *Celtis philippensis* justified its folkloric use in treating infections. Moreover, good activity on multidrug resistant wound pathogens highlights its potentials as a source of antimicrobial agents to treat wounds infected with MDR pathogens.

Keywords: *Celtis philippensis*, multidrug resistant pathogens, antibacterial, antioxidant, wounds.

INTRODUCTION

Wound infection is one of the most common diseases in developing countries because of poor hygiene causing morbidity and mortality amidst the populace.[1,2] Most community acquired wound infections are polymicrobial in nature of which 25% are caused by Staphylococcus aureus while Gram-negative bacteria like *Pseudomonas aeruginosa, Klebsiella pneumoniae, Peptostreptococcus* are also implicated.[3,4] The risk of developing a wound infection particularly with multi-drug resistant strains of pathogenic bacteria is on the increase.[5] In a 5-year surveillance of wound infections study conducted in Edo State, Nigeria, the overall prevalence of wound infection was 70.1% with *S*. aureus and *P*. aeruginosa as the prevalent organisms all through the years of study.[6] Many studies carried out in different parts of Nigeria showed that *P*. aeruginosa, *S*. aureus, Klebsiella species, E. coli and Proteus species were the most common pathogens isolated from wounds.[7] Today, due to high cost of effective antibiotics and the prevalent antibiotic resistance among microbial strains worldwide, about 60-85% of the population of developing world rely either on herbal or indigenous forms of complementary and alternative medicines for treatment or management of their infections and diseases.[8,9,10] Moreover, free radicals and reactive oxygen species (ROS) have been implicated in many ailments including wound infection. Medicinal plants with antioxidant activity can play a role in the removal or scavenging of toxic free radicals from human body.[11] Medicinal plants have been used since time immemorial for treatment of various ailments of skin and dermatological disorders especially cuts, wounds and burns.[12]

Celtis philippensis Blanco (Ulmaceae) also known as Celtis wigthii Planch in Nigeria, is a small forest tree widely distributed in tropical Africa, Asia and Australia. Among other Celtis, it has characteristic leaves that are toothed and prominently 3-nerved;[13] found in Nigeria and commonly distributed across the region from Senegal to Cameroons. Medicinally, its leaf-sap is used in treating cutaneous and subcutaneous parasitic infection and in India to treat diabetes. The genus *Celtis* is of great medicinal importance in the family Ulmaceae (or Cannabaceae); species worthy of note is Celtis australis L., (Mediterranean hackberry) whose decoctions of the leaves and fruits were used to astringe the mucous membrane in peptic ulcers, diarrhoea, and dysentery and as a remedy for heavy menstrual bleeding. Also, Celtis occidentalis L., of which native Americans used decoctions prepared from the bark as an aid in menses and to relieve sore throat and used the wood extract in treating jaundice.[14] Others are Celtis africana Burm. f, and *Celtis* iguana (Jacq.) Sarg. Due to the immense ethnomedicinal claims and the antimicrobial activities of allied species, we thereby investigated and hereby report the phytochemical constituents, antioxidant and antibacterial activities of *Celtis philippensis* extracts on multidrug resistant wound pathogens from three Nigerian hospitals.

METHODS

Plant material

Fresh stems of *Celtis philippensis* were collected from the Botanical Garden of the University of Ibadan. The plant material was identified and authenticated in Forestry Research Institute of Nigeria (FRIN) with a deposited voucher specimen number FHI NO: 109993. The stems were air-dried, milled into coarse powders and extracted with 40% methanol using a Soxhlet extractor. The extract was evaporated under reduced pressure, dried and stored at 4oC for use.

Test organisms

Thirty-two (32) clinical isolates comprising of *Staphylococcus aureus* (14), *Pseudomonas aeruginosa* (10) and *Klebsiella pneumonia* (8) from wound infections were collected from Medical Microbiology departments of three Nigerian hospitals; University College Hospital, Ibadan, University of Ilorin Teaching Hospital, Ilorin and Obafemi Awolowo Teaching Hospital, Ile-Ife, Nigeria. The identity of the isolates was confirmed by standard cultural and biochemical tests. Typed strains of *Staphylococcus aureus* (ATCC 25923) and *Pseudomonas aeruginosa* (ATCC 15442) used as reference standards were obtained from Pharmaceutical Microbiology laboratory, University of Ibadan. They were all maintained on agar slants at 4oC prior to use.

Antibiogram Determination

The susceptibility testing of the isolates to standard antibiotics was performed using Kirby-Bauer disc

diffusion method. The antibiotics used were selected based on the type of wound pathogen (whether Gram-positive or Gram-negative) and according to drug treatment of infections caused by the pathogen. Therefore, the following antimicrobial agents were used as follows: For Staphylococcus aureus, AMX= amoxicillin, OFL= ofloxacin, STR=streptomycin, CEF= ceftriaxone, GEN= gentamicin, PEF= pefloxacin, COT=cotrimoxazole, CPX= ciprofloxacin, ERY= erythromycin, CHL= chloramphenicol; OX= oxacillin, FOX= cefoxitin; for Psedomonas aeruginosa: CXM=cefotaxime, OFL=ofloxacin, AUG=augmentin, NIT=nitrofurantoin, CPR=ciprofloxacin, CAZ=ceftazidine, CRX=cefuroxime, GEN=gentamicin, IMP= imipenem; for Klebsiella pneumoniae: CXM=cefotaxime; OFL=ofloxacin, AUG=augmentin; NIT=nitrofurantoin; CPR=ciprofloxacin, CAZ=ceftazidine; CRX=cefuroxime; GEN=gentamicin; IMP= imipenem; CTR= ceftriazone. The zones of inhibition around each disc was recorded and interpreted by CLSI guidelines.[21]

Antimicrobial Screening

Using agar-well diffusion method as described by Perez *et al*.,[15] a 0.1ml of a two-fold dilution of overnight broth culture of each bacterium (containing 1.0 x 10^8 cfu/ml, equivalent to the turbidity of 0.5 McFarland standard) was seeded into 20ml nutrient agar. 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. Control wells containing gentamicin (10ug/ml), Ceftriaxone (30 ug/ml) and 40% methanol were used as positive and negative controls respectively. A pre-incubation period of about 1hour at 40C was allowed for the diffusion of extracts and test solutions before incubation. Bacterial plates were incubated at 370C for 24hours after which the zones of inhibition were measured.

Determination of Minimum Inhibitory Concentration (MIC) of the Plant Extracts

The minimum inhibitory concentration of the methanolic plant extracts were determined by the agar dilution method. The plant extracts were prepared in graduated decreasing serial two-fold dilutions to produce the following concentrations:20, 10, 5, 2.5, 1.25, 0.625, 0.312 and 0.156mg/ml.Each bottle containing 18ml of nutrient agar was melted and cooled to 45-50°C and 2ml of each of the concentration or dilution was added. The bottle was mixed properly and poured in a Petri dish. The agar was then allowed to set firmly. The organisms were inoculated by streaking on the plates which were then incubated at 370C for 24 hours. The MIC was taken as the minimum concentration of plant extracts that inhibited discernible bacterial growth in the plates.[16]

Determination of Minimum Bactericidal Concentration (MBC) of the 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 370C for 24 hours. The MBC was recorded as the lowest concentration of antibiotic inhibiting all growth.[16]

Determination of Antioxidant Potentials

Catalase Activity

The activity of catalase (CAT) was measured according to the method of Aebi,[17] as the disappearance of hydrogen peroxide at 240 nm in a reaction medium containing 1800 µl of 50 mM phosphate buffer (pH 7.0). 180 µl of 300 mM H2O2, and 20 µl of methanolic extract (1:50 dilution). The reaction was monitored for 2 mins (10 intervals), at 240 nm using a UV-visible spectrophotometer (Cecil CE 7200 spectrophotometer, Cecil instrument limited, Milton Technical Centre, England) and expressed as µmol of H2O2 consumed/min/mg extract).

DPPH Radical Scavenging Activity

The methanolic extract (100 µl, 1mg/ml) was added to 3.9ml of DPPH solution (0.025g/L) and the reactants were incubated at 25oC for 30 mins. Instead of extract, a positive control of AsA was used. The mixture was shaken and allowed to stand in the dark at room temperature for 35 mins. Blank methanol and ascorbic acid (25 - 0.78 pg/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[18] 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

Hydrogen Peroxide Radical Scavenging (HPRS) Activity

One milimetre of extract (250µg/ml) was mixed with 2.4ml of 0.1 M phosphate buffer (pH 7.4) and then 0.6 of a 43Mm solution of H202 in the same buffer were added.[19] After 40 mins, the absorbance of reaction mixture was taken at 230nm against a blank solution (phosphate buffer without H202) using the UV-spectrophotometer (Cecil CE 7200 spectrophotometer, Cecil instrument limited, Milton Technical Centre, England). Percentage scavenging of H202 was calculated with Ascorbic acid as control.

% inhibition (radical scavenging %) = (AC-AS) /Ac×100

Lipid Peroxidation Inhibition (LPI) Using Thiobarbituric Acid Method

Extracts (2ml) and standard solutions (2ml) of the extract were added to 1ml of 20% aqeous trichloroacetic acid and 2 ml of 0.67% aqeous thiobarbituric acid. After boiling for 10mins, the samples were cooled. The tubes were centrifuged at 3000 rpm for 30 min. Absorbance of the supernatant was measured at 532 nm in a spectrophotometer[20] (Cecil CE 7200 spectrophotometer, Cecil instrument limited, Milton Technical Centre, England). Percentage scavenging of H202 was calculated with Ascorbic acid as control.

% inhibition (radical scavenging %) = (AC-AS) /Ac×100.

AS= Absorbance of the control; AS= Absorbance of the sample.

Statistical analysis

Data were analyzed using Analysis of Variance (ANOVA) at 1% level of significance. A difference was considered statistically significant at $p < 0.05$ (R programme was used).

RESULTS

The antibiogram of the clinical isolates of the test bacteria showed the resistance levels of the organisms as shown in Tables 1, 2 and 3 for *Staphylococcus aureus*, *Pseudomonas aureus* and *Klebsiella pneumoniae*, respectively. For ease of comparison and interpretation, the percentage resistance to each antimicrobial agent is presented as a summary in Table 4. All the zones of inhibition of the antimicrobial agents on each bacteria strain were classified into Resistance ®, Intermediate (I) and Susceptible (S) according to CLSI, 2007.[21] The in vitro antimicrobial screening of the methanolic extracts on *Staphylococcus aureus, Pseudomonas aureus* and *Klebsiella pneumoniae* isolates used in this study were recorded as shown in Table 5. Both concentrations used (20mg/ml and 10mg/ml) of the methanolic extracts showed a clear zone of inhibitions comparable to those shown by the positive controls (ceftriaxone and gentamicin). The diameter of zone of inhibition ranged between 16-35mm at 20mg/ml and 13-25mm at 10mg/ml. The minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC) of the methanolic extract (shown in Table 6) ranged between 0.625-5 mg/ml for K. pneumoniae isolates, 0.625mg/ml for P. aeruginosa isolates and 1.25-2.5mg/ml for *S*. aureus isolates. The minimum bactericidal concentration of the methanolic extracts varies between 1.25-5mg/ml for *S. aureus* and *K. pneumoniae* isolates, 0.625-5mg/ml for P. *aeruginosa* isolates. The extract had a higher MIC values for the *S*. aureus test isolates than that for K. pneumoniae and *P*. aeruginosa test isolates. The result of the Analysis of variance (ANOVA) shows that there is a significance difference among the variables measured at 1% level of significance on the *P. aeruginosa* isolates but no significance difference among the *S. aureus* and *K. pneumoniae* isolates when compared by Tukey's post-test in a One-way ANOVA analysis. The results of three antioxidant assays were presented in Figure 1 as a bar chart in comparison with ascorbic acid control.

ANTIBIOGRAM OF CLINICAL ISOLATES FROM WOUND INFECTION

Table 1: Antibiotic Susceptibility of *Staphylococcus aureus* Isolates

Isolate/Abs	AMX	OFL	STR	CHL	CEF	GEN	PEF	COT	CPX	ERY	OX	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	I	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

Key: R= Resistant; AMX= amoxicillin, OFL= ofloxacin, STR=streptomycin, CEF= ceftriaxone, GEN= gentamicin, PEF= pefloxacin, COT=cotrimoxazole, CPX= ciprofloxacin, ERY= erythromycin, CHL= chloramphenicol; OX= oxacillin, FOX= cefoxitin

Table 2: Antibiotic Susceptibility of *Pseudomonas aeruginosa* Isolates

Isolate	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

Key: R=Resistant, CXM=cefotaxime, OFL=ofloxacin, AUG=augmentin, NIT=nitrofurantoin, CPR=ciprofloxacin, CAZ=ceftazidine, CRX=cefuroxime, GEN=gentamicin, IMP= imipenem

Table 3: Antimicrobial Susceptibility of *Klebsiella pneumoniae* Isolates

ISOLATES	CRX	CPR	NIT	AUG	OFL	CXM	GEN	CTR	IMP	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

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

Table 4: Summary of Antimicrobial Resistance of *Staphylococcus, Pseudomonas* and *Klebsiella* species

	Pseudomonas aeruginosa	*Klebsiella pneumonia*	*Staphylococcus aureus*
Antibiotics	Resistance (%)	Resistance (%)	Resistance (%)
Cefotaxime	100.00	87.50	_
Augmentin	100.00	87.50	_
Nitrofurantoin	100.00	62.50	_
Cefuroxime	100.00	100.00	_
Ceftazidime	60.00	62.50	_
Imipenem	100.00	25.00	_
Gentamicin	80.00	37.50	85.71
Ofloxacin	80.00	25.00	64.29
Ciprofloxacin	80.00	_	50.00
Ceftriaxone	_	62.50	35.71
Pefloxacin	_	_	57.14
Streptomycin	_	_	57.14
Erythromycin	_	_	92.86
Cotrimoxazole	_	_	85.71
Chloramphenicol	_	_	64.29
Amoxicillin	_	_	100.00
Oxacillin	_	_	100.00
Cefoxitin	_	_	92.86

Key: – = Not tested

Table 5.0:Antimicrobial Activity of *Celtis philippensis* stem extract on Wound Pathogens

	Diameter(mm) of Zone of inhibition of Extract/Controls on Test Bacteria			
	Celtis philippensis stem extract		Gentamicin	Ceftriaxone
Isolates	20mg/ml	10mg/ml	10 µg	30 µg
S1	19	14	23	25
S2	17	14	25	I,18
S3	20	16	24	30
S4	20	16	25	30
S5	21	15	15	I,20
S6	20	13	R,8	I,18
S7	20	18	R ,8	20
S8	18	17	R,8	I,15
S9	20	20	R8	R,8
S10	25	25	20	30
S 11	24	22	32	I,20
S12	27	25	26	32
S13	18	16	30	25
S14	30	27	25	I,20
P1	22	20	23	I,16
P2	21	18	R,8	R,8
P3	22	20	R,8	R,8
P4	25	20	R,8	R,8
P5	25	20	24	I,18
P6	28	25	R,8	R,8
P7	24	22	R,8	R,8
P8	22	19	R,8	R,8
P9	26	20	R,8	I,20
P10	26	22	17	25
K2	20	16	20	30
K3	20	16	20	22
K4	26	16	20	24
K5	28	20	21	26
K6	20	16	I,14	I,20
K8	16	14	22	20
K16	35	22	20	30

Keys: S1 – S14 = *Staphylococcus aureus*, P1 – P10 = *Pseudomonas aeruginosa*, K2 – K16 = *Klebsiella pneumoniae*

Table 6.0: MIC and MBC of Celtis *philippensis* Extract on Test Bacteria

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

Keys: S1 – S10 = *Staphylococcus aureus*, P1 – 10 = P *Pseudomonas aeruginosa*, K3 – K18 = *Klebsiella pneumoniae*

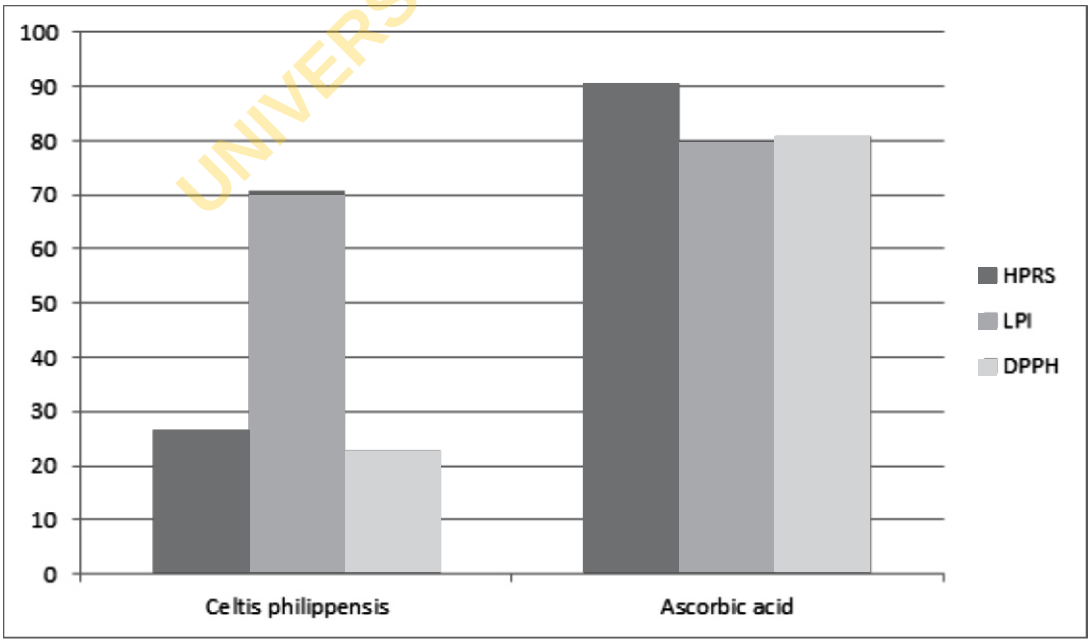

Fig. 1: Antioxidant activities (%) of Celtis philippensisby HPRS, LPI and DPPH methods compared with that of ascorbic acid reference standard

DISCUSSION

The antibiogram showed that most of the tested wound pathogens were multidrug resistant (MDR), having shown resistance to three or more antimicrobial agents belonging to different classes. The Pseudomonas isolates showed 100% resistance to augmentin, cefuroxime, ceftriaxone, nitrofurantoin and imipenem; the most active drug being ceftazidime (40%) and fluoroquinolones (20%). Actually, 60% of the *Pseudomonas* showed 100% resistance to all tested antibacterials. These type of MDR *Pseudomonas aeruginosa* strains were also reported for Egyptian hospitals by Ahmed *et al*.,[22] but this is at higher levels, which is an indication of increasing resistance. In this study, the most active drug on the *Pseudomonas* isolates was ceftazidime which is similar to that reported by Diab *et al*.,[23] here the activity of ceftazidime was 40% while theirs was 54%,[23] which also indicated increasing resistance. The Klebsiella strains were relatively more susceptible to the chemotherapeutic agents, but with tendency for more to acquire resistance with time. The Staphylococci also showed a high-level resistance which is consistent with that obtainable with methicillin resistant *Staphylococcus aureus* (MRSA), vancomycin intermediate Staphylococcus aureus (VISA) and vancomycin resistant *Staphylococcus aureus* (VRSA). The Staphylococcus aureus strains were 93-100% resistant to the ?-lactam drugs but were most susceptible to ceftriaxone 35%. As MRSA is the commonest aetiology of nosocomial wound infection,[24] its eradication has become more difficult with the emergence of VISA and VRSA.The antibiogram has clearly revealed that the wound pathogens used were actually MDR which enables us to appreciate the significant broad-spectrum activity of the plant's extracts on the pathogens.[24]

The role of medicinal herbs in treating wound infection dated from Biblical era when extract from Fig tree, *Ficus carica* (Moraceae) was used to heal the boil on king Hezekiah.[25] With increasing antimicrobial resistance worldwide, the search for alternative therapy among natural herbs is essential and must be taken from empirical to scientific documentation. In this study, it behoves us to report that the extract of *Celtis philippensis* was very active on MDR wound pathogens. The Gram-negative bacteria were more susceptible to the plant extracts than Gram-positive bacteria which contradicts some previous reports that plant extracts were more active against Gram-positive bacteria than Gram-negative bacteria.[26,27] Pseudomonas aeruginosa isolates which were highly resistant to the antimicrobials were highly susceptible to the extract. The MDR Staphylococcus aureus, Pseudomonas aeruginosa and Klebsiella pneumonia were all susceptible to the extract at 10 and 20 mg/ml. The minimum inhibitory concentration (MIC) of the extract that inhibited the antibiotic resistant organisms are 0.625mg/ml (for Pseudomonas and four of the Klebsiella) and 2.5mg/ml for the *S. aureus* which were the least susceptible. MIC Index of the extract (MBC/MIC) was less than 4 indicating bactericidal activity especially of the stem extract.[28] The *S. aureus* test isolates were not very susceptible to the extract. Ability of herbal extracts to overcome resistant pathogens can be attributed to the presence of multiple antimicrobial compounds, synergistic or additive effect among bioactive compounds and diverse nature of constituents that organisms cannot readily produce genes against.

The roles of antioxidants in wound healing are well documented. Natural antioxidants in medicinal plants occur in form of phenolics, flavonoids and polyhydroxyl compounds which are able mop-up or scavenge the reactive radicals and oxygen species, thereby enhancing tissue repair and promote wound healing. According to Dudonnei *et al*.,[29] antioxidant activities of medicinal plants must be evaluated by at least two methods in order to take into account different modes of action of a given antioxidant.[29] The antioxidant activities revealed that the extract showed high potential in the lipid per-oxidation inhibition and catalase activity. Its activity on hydrogen peroxide scavenging and DPPH radical scavenging were relatively low compared with the ascorbic acid standard. Phytochemicals present in the extract 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 532 nm. This led to reduction of adduct formation indicating their lipo-protective potential. The extract also showed the capability to catalyze the

dismutation of hydrogen peroxide in water and oxygen.

CONCLUSION

The extract was active against both Gram-positive and Gram-negative bacterial isolates tested and this may indicate a broad spectrum of activity. The results of the study support the traditional application of *C. Philippensis* in treating bacterial, cutaneous and subcutaneous parasitic infections. The susceptibility of all the test organisms (MDR), including *P. aeruginosa* that has resistance against many antibiotics goes further to prove that the plant has potentials as alternative source of antimicrobial agent in overcoming the problems of resistance to chemotherapeutic drugs. *Celtis philippensis* stem has also been revealed as a source of antioxidant agents. It is therefore desirable to isolate and characterize the antimicrobial and antioxidant agents from *C. philippensis.*

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